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Spatial Memory in Black-capped Chickadees: Studies of Adult Hippocampal Neurogenesis and Win-Shift/Win-Stay Spatial Search

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Abstract

Two cognitive adaptations were studied in Black-capped chickadees through tests of adult hippocampal neurogenesis and Win-shift/Win-stay spatial search. Neurogenesis has been proposed to aid memory, therefore it was hypothesized that birds with decreased neurogenesis would perform poorer than controls in hippocampal-dependent spatial working and reference memory tasks followed by a reversal. Subjects with decreased neurogenesis, caused by the neurotoxin MAM, reversed slower than controls, suggesting that neurogenesis may contribute to differentiating similar memories, although this effect was nonsignificant. Win-shift/Win-stay foraging behavior is an adaptation to the replenishing and depleting nature of food. Since chickadees forage on food that depletes quickly and slowly, it was hypothesized that chickadees would change their foraging strategy in response to reward contingency in a spatial working memory task. I found that chickadees did not respond to reward contingency and instead relied on individual preferences. Sweeping general models do not always apply to complex foraging birds.

Keywords

Black-capped Chickadees, Spatial Memory, Working Memory, Reference Memory, Hippocampus, Neurogenesis, Adult Hippocampal Neurogenesis, Methylazoxymethanol Acetate, Win-Shift/Win-Stay, Foraging, Stereology

Co-Authorship Statement

The experiments conducted in my thesis were carried out under the supervision of Dr. David Sherry. The study presented in Chapter 2 was done in collaboration with Dr. David Sherry. The study presented in Chapter 3 was done in collaboration with Caroline Strang who helped design the experiment, and Christopher Course who helped analyze data. Both Caroline and Christopher aided in data collection and provided helpful feedback with analyses and writing. The quality of research presented in the current thesis benefitted greatly from the insight of fellow Advanced Facility for Avian Research lab members Caroline Strang, Shannon Mischler, Christopher Course, Robert Martin, and Madeleine Brodbeck. This thesis also benefitted from the extensive feedback from my committee members Dr. Peter Ossenkopp, Dr. William Roberts, and my advisor Dr. David Sherry, most importantly.

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List of Abbreviations & Nomenclature

APH	Area Parahippocampus
CAP	Hippocampal Cap
CA1	Area CA1 of the hippocampus
CA2	Area CA2 of the hippocampus
CA3	Area CA3 of the hippocampus
CE	Coefficient of Error
D	Dorsal
DCX	Doublecortin
DG	Dentate Gyrus
g	Grams
h	Hours
HA	Hyperpallium Apicale
HVC	Higher Vocal Control Centre
i.m	Intramuscular
L	Lateral
m	Meters
M	Moles
mm	Millimeter
mg/kg	Milligrams per killogram
MAM	Methylazoxymethanol Acetate
min	Minutes

NONSVZ	Non-Subventricular Zone
PBS	Phosphate buffered saline
PBS/T	Phosphate buffered saline triton
pH	Potential Hydrogen
QMR	Quantitative Magentic Resonance
s	Seconds
SVZ	Subventricular Zone
μm	Microns
V	Ventricle
“	Inches

1.1 SPATIAL MEMORY IN BLACK-CAPPED CHICKADEES: STUDIES OF ADULT HIPPOCAMPAL NEUROGENESIS AND WIN-SHIFT/WIN-STAY SPATIAL SEARCH

Introduction

Spatial memory is essential for human and animal survival. We must be able to remember the locations of food, shelter, and dangers in our environment in order to survive and evolve. The hippocampus is a brain structure associated with spatial memory and cognition (Krebs et al., 1989; Broadbent & Colombo, 2000; Clelland et al., 2009). Black-capped chickadees are food-storing passerines that are able to keep track of many stored food locations and rely on their spatial memory to find food caches (Hoshooley & Sherry, 2007; Sherry, 1984). Black-capped chickadees also experience adult hippocampal neurogenesis, the production of new functional neurons in the brain (Barnea & Nottebohm, 1994). The function of adult hippocampal neurogenesis in Black-capped chickadees and other species is not well understood. In a food-storing species it has been hypothesized to act as a cognitive adaption to the high demands of remembering many food sites on spatial memory (Sherry, 1984; Tomback, 1980). Sensitivity to the depleting and replenishing nature of food sources is another suggested cognitive adaptation in chickadees. The present research was designed to provide insight into the influence of adult hippocampal neurogenesis on spatial memory for the locations of food, and to provide additional information about foraging strategies in Black-capped chickadees through a win-shift/win-stay spatial search task.

1.2 Spatial Memory

Spatial memory is memory for locations in an environment, such as the locations of shelter and food. Spatial memory may be a cognitive adaption that allows for the most conservative use of resources (Sherry, 1984; Tomback, 1980). For example, it is beneficial for an animal to be able to return quickly to a shelter to avoid predation. Spatial working memory is memory for the distinctive features of an experience, such as your last move in a game of chess or where an animal last found food, while spatial reference memory is memory for the stable features of an experience, such as how each piece can move or where your shelter is located (Olton, Becker & Handelmann, 1979). Chickadees have impressive spatial memory and are able to keep track of thousands of stored food locations (Sherry, 1984; Healy & Hurly, 2004). The chickadee hippocampus plays a critical role in spatial cognition (Hampton & Shettleworth,

1996). Neurogenesis and apoptosis both occur in the chickadee hippocampus into adulthood, but it remains unknown whether a new neuron becomes functional in the same position, and with the same capacity, as the one it replaces (Barnea & Pravosudov, 2011). Chickadees are an excellent model for the study of spatial memory and neurogenesis because they have a specialized hippocampus with high levels of neurogenesis (Hoshooley & Sherry, 2007). Chickadees have been used successfully as models for studying neuroproliferation in the past (Barnea & Pravosudov, 2011; Hall, Delaney & Sherry, 2014).

1.3 Avian Hippocampus and Adult Hippocampal Neurogenesis

Hippocampal size is significantly correlated with the accuracy of food-storing and recovery behavior in birds (Sherry, 2011). Food-caching species appear to have more intense hippocampal neurogenesis than non-caching passerine species (Hoshooley & Sherry, 2007; LaDage et al., 2010). The hippocampus is known to be essential for learning, memory and behavioural inhibition (Clelland et al., 2009; Deng, Aimone & Gage, 2010; Scarf et al., 2014). It is important for the formation of episodic and spatial memory, and emotional behavior in humans (Deng et al., 2010). New neurons are recruited into the hippocampus of adult chickadees (Hoshooley & Sherry, 2007). Chickadees undergo a number of behavioural and ecological changes during the fall in preparation for winter that are correlated with a peak in adult hippocampal neurogenesis and food storing. Scatter caching species need to form new memories about new caches constantly, so perhaps neurogenesis is adaptive by providing new neurons for new memories. It remains unclear, however, whether increased neurogenesis is simply a consequence of higher memory use or whether increased neurogenesis causally enhances memory function (Barnea & Pravosudov, 2011).

For decades it was believed that neurogenesis did not occur into adulthood despite the early work of Das and Altman (1972) showing that neurogenesis persists in the subgranular zone of the hippocampus beyond development. Later research conducted by Goldman and Nottebohm (1983) uncovered neuronal precursors in the song control nuclei of canaries. They found many labeled HVC neurons after thirty days when birds were injected with the birth-date marker tritiated thymidine, but not after one or two days. After the shorter time, many new neurons were labeled on the wall of the ventricular zone (VZ) and the researchers concluded that new neurons were born in the VZ and migrate into the telencephalon where they settle and differentiate. Today we know that most neurons are actually born quite far from where they will ultimately

reside (Barnea & Pravosudov, 2011). Adult neurogenesis has now been discovered in the hippocampi of mammals (Gross, 2000; Gould & Gross, 2002; Eriksson, et al., 1998), and birds (Nottebohm, 1981; Goldman & Nottebohm, 1983; Barnea & Nottebohm, 1996) among other classes. In mammals, new neurons are generated in the subgranular zones and migrate to the dentate gyrus of the hippocampus and olfactory bulb (Kaslin et al., 2008; Eriksson, et al., 1998). In the avian brain, new neurons are generated along the walls of the lateral ventricles and migrate radially throughout the brain extending beyond the hippocampus and olfactory areas (Kaslin et al., 2008; Vellema et al., 2010). The avian and mammal hippocampi are considered to be homologous (See Figure 1.1; Jarvis et al., 2008; Barnea & Pravosudov, 2011; Atoji & Wild, 2004; Broadbent & Colombo, 2000). It is suggested that the V shaped region, or "darkly staining V" in Nissl stained tissue in birds is homologous to the dentate gyrus structure in mammals (Atoji & Wild, 2004). Cameron et al., (1993) developed the immunohistochemical methods that made it possible to label new granule cells and quantitatively measure neurogenesis in the brain.

1.4 Pattern Completion and Pattern Separation in the Hippocampus

Today we know that memory retrieval relies on pattern completion: reactivation of the pattern of cellular activity that occurred during encoding (Frankland, Köhler & Josselyn, 2014), and that successfully distinguishing between two memories during encoding relies on pattern separation: differentiating the cellular activation associated with two memories by reducing the average overlap of brain activation between them (Treves & Rolls, 1994). Considering that the hippocampus must be capable of using degraded or noisy recall cues to receive previously stored activity patterns (Hunsaker & Kesner, 2013), pattern separation has also been defined as the formation of distinct and orthogonal representations of mnemonic information (Clelland et al., 2009). In 1971 Marr wrote that little information about a single learnt event is required to provoke its recall, and proposed a mathematical model of a pre-existing structure responsible for direct storage of memory associations. The hippocampus serves two primary functions: (1) acting as a competitive learning network that reduces the degree of overlap among activity patterns to facilitate storage with minimal interference by other activity patterns, and (2) to serve as an auto association network that is capable of recalling stored activity patterns from partial cues (Marr, 1971; Kesner et al., 1987). The hippocampus facilitates learning and recall of information through pattern separation and completion (Hunsaker & Kesner, 2013), both of which are influenced by changes in neuroproliferation.

Hunsaker and Kesner (2013) have suggested using what they call an Attribute Model for the study of pattern separation and pattern completion processes. They suggest using an event-based memory system in episodic memory processing with short-term retrospective memory processes such as working memory. The event-based system is one that includes pattern separation processes that are essential for encoding information. In contrast, the knowledge-based memory system is used in retrieval of long-term representations of information previously encoded by the event-based system, such as a reference memory, and uses pattern completion processes.

1.5 Function and Manipulation of Adult Hippocampal Neurogenesis

Neurogenesis can be manipulated experimentally and often causes changes to pattern completion and pattern separation processes. Techniques for increasing neurogenesis are of great interest to those hoping to improve hippocampal functions. Environmental enrichment, exercise (Van Praag, Kempermann & Gage, 2000) and antidepressants (Malberg et al., 2000) increase neurogenesis. The learning of hippocampal dependent tasks is a major regulator of adult hippocampal neurogenesis, and increases the number of newborn neurons in the hippocampus by promoting their survival (Deng et al., 2010). In mice, increased neuroproliferation improves performance in a cognitive task in which two similar contexts need to be distinguished (Sahay et al., 2011), leading to the conclusion that an increase in neurogenesis facilitates pattern separation. In rats, learning the Morris water maze promotes the survival of new cells born a week prior to training, and induces apoptosis of cells born in the early stages of training (Deng et al., 2010). Stimulating adult neurogenesis may also be a novel therapeutic strategy for treating anxiety disorders, as rats contextual fear discrimination task performance also improves as neurogenesis increases (Sahay et al., 2011).

Decreasing neurogenesis is another way to study learning and memory. There is evidence that newborn neurons may be necessary for normal pattern separation in the dentate gyrus of adult mice (Clelland et al., 2009). When neurogenesis was stalled, researchers found specific impairments in spatial discrimination on a radial arm maze and memory touch screen task. The ability to pattern separate, or differentially encode small or weak changes derived from increasingly similar or interfering inputs, is particularly important for the accuracy of memory encoding (Clelland et al., 2009) and may require new neurons (Deng et al., 2010).

There is a hypothesis that young hippocampal cells mediate pattern separation, while old ones facilitate pattern completion (Nakashiba, et al., 2012). Transgenic mice born with the output of old granule cells inhibited showed enhanced pattern separation and reduced pattern completion between similar contexts that was abolished by ablation of young granule cells (Nikashiba et al., 2012). New neurons may be recruited into existing neural circuits and directly involved in all stages of memory processing (Schneider & Gage, 2004), or new neurons primarily may be necessary to avoid interference when new information is being learned (Wiskott, Rash & Kempermann, 2006; Deng et al., 2010).

Another theory suggests that adult neurogenesis provides a "neurogenic reserve" that allows the brain to remain flexible in learning by recruiting new neurons from this reserve when there is new information to be learned (Kempermann, 2008). This hypothesis predicts that new neurons become incorporated into existing neural circuits only when there is a need for new learning. Kempermann's theory may explain the mixed findings in this area of research. Importantly, his theory states that learning deficits should be observed only when the reserve of "ready" neurons is depleted, therefore, some subjects could fail to show behavioural changes on a task after neurogenesis manipulations because they have a reserve of new neurons ready to be incorporated when required.

Many researchers associate deficits in pattern separation and decreased neurogenesis with neurocognitive aging (Hunsaker & Kesner, 2013). Unfortunately for humans, neurogenesis decreases with age and is associated with age-related cognitive decline and pathological aging (Wesnes, 2010; Rosenzweig & Barnes, 2003; Small et al., 2004). A model of the information-processing circuit of the aging hippocampus suggests that changes in aging strengthen existing memories at the cost of processing new ones, with information already stored becoming the dominant pattern of the hippocampus (Wilson et al., 2006). Therefore, it is beneficial to study and measure the effects of decreased neurogenesis with the goal of understanding its function. Understanding the function of neurogenesis could allow us to develop behavioral or contextual therapies to prevent the possible detrimental effects of reduced neurogenesis on learning and memory.

1.6 Black-capped Chickadees as a Model to Study Neurogenesis

Rats, mice and birds are all common models for studying neurogenesis. Barnea and Pravosudov (2011) have suggested that birds may be an ideal model for studying neurogenesis

because they permit a combination of evolutionary, comparative and neuroethological approaches. Many songbirds sing songs with a seasonal variation associated with neuronal turnover in song control nuclei regulated by neurogenesis. Food-caching birds, like chickadees, use memory-dependent behavior in learning the locations of scattered food caches. They have large hippocampi and experience neurogenesis linked to spatial learning. Blocking neurogenesis results in impaired spatial memory when cues to be remembered have little spatial separation, but not when cues have large spatial separation (Clelland et al., 2009), suggesting that new neurons may be needed for pattern separation. The naturally occurring memory based behavior of chickadees, and that they can be studied in the wild and in the laboratory, make them ideal for investigation of the neurological processes that underly learning (Barnea & Pravosudov, 2011).

Neurogenesis can be manipulated experimentally in chickadees using methylazoxymethanol acetate (MAM), a neurotoxin that disrupts DNA synthesis and suppresses cell proliferation in the brain without significantly changing measures of body composition (Hall et al., 2014). In rats, MAM has decreased neuroproliferation by 84% (Shors et al., 2002) and significantly altered hippocampal functions like spatial memory. In Black-capped chickadees, MAM has decreased neuroproliferation by 46% (Hall et al., 2014) and caussd deficits in spatial reversal learning.

Adult hippocampal neurogenesis was only recently discovered (Altman, 1962; Goldman & Nottebohm, 1983). It is a process that has profound repercussions for pattern completion and pattern separation – two processes that mediate memory retrieval and encoding. A number of hypotheses for the function of adult neurogenesis specific to birds have been suggested. These hypotheses include: (1) that adult neurogenesis is a epiphenomenon remaining from development that serves no particular function; (2) new neurons are directly involved in learning; and (3) adult neurogenesis is necessary for the replacement of old neurons that have become damaged after intense use (Wilbrecht & Kirn, 2004).

Neurogenesis has implications for depression, Alzheimer's disease and schizophrenia in humans (Barnea & Pravosudov, 2011). However, in our search to understand the function of adult neurogenesis, we should not assume that the answers we find will indisputably advance methods for brain repair of previously intractable problems (Gould & Gross, 2002). There are still many questions to be asked and alternative hypothesis to be tested in this area. We must exercise caution and objectivity when suggesting we can alter the human brain. This area of

research will become more consistent as we replicate valid findings and test competing hypotheses that are all a small part of the large puzzle that is adult neurogenesis. Understanding neurogenesis and spatial memory in chickadees is an important step in understanding the mechanisms involved in the impressive memory systems of food-storing birds.

1.7 Avian Food Storage, Foraging and Memory

Many birds, including nutcrackers, jays, tits, and chickadees, rely on stored food to survive the winter and raise their young (Tomback, 1977; Roberts, 1979). Memory for the locations of food with information about the type of food, and whether it has been harvested or pilfered, increases the effectiveness of cache retrieval (Sherry, 1984). Black-capped chickadees have memory for what, where and when (Feeney & Roberts, 2009). They are able to remember what kind of food they encounter, where they encounter it, and when they encounter it, independently of food caching or retrieval. This ability is not unique to Black-capped chickadees and has been studied in scrub jays (Clayton & Dickinson, 1998), magpies (Zinkivskay et al., 2009), pigeons (Skov-Rackette et al., 2006), rats (Babb & Crystal, 2005), mice (Sere et al., 2005), meadow voles (Ferkin et al., 2008), gorillas (Schwartz et al., 2005), rhesus monkeys (Hampton et al., 2005), and Yucatan minipigs (Kouwenberg et al., 2009), among other species. In chickadees, this kind of memory is important for food storage. Chickadees are able to accurately relocate cached food, recall which cache sites have been emptied or discovered empty, and recall which kind of food is located at each cache site (Sherry, 1984).

Caching birds have enhanced memory and an enlarged hippocampus due to the selection pressure for superior memory that is required to recover previously cached food (Krebs et al., 1989; Sherry et al., 1989). Chickadees scatter cache their food and do not larder cache. This means they allocate small amounts (1 or 2 pieces) of food to many cache sites instead of allocating many pieces of food to only one or two cache sites. The cost of scatter caching is greater because it requires a mechanism, like spatial memory, to facilitate the recovery of stored food in many locations. Larder caching is riskier, since a pilfered cache site would result in a large loss. There is evidence that food-storing birds use spatial memory to recover their caches (Tomback, 1980; Smulders & DeVoogd, 2000). Local cues (Vander Wall, 1982), microtopography (Balda, 1980), and visual landmarks (Bossema, 1979) are all possible cues used to remember locations. Spatial memory for relocating caches is a selective advantage (Andersson & Krebs, 1978). Chickadees are able to remember where they have cached food, and

visit sites where they have stored food, significantly more often than sites where they have not stored food. They do this without engaging in a random or preferred search path, seeing or smelling the food (Sherry, 1984).

Chickadee memory for the locations of cached food is more accurate than memory for the locations of found food (Baker at al., 1988). Watching another bird store seeds does not help the watcher find those seeds, even though the same watcher has no problem recovering their own cached food. The perceptual and motor experience of finding food, carrying it to a location, and storing it there, may be necessary to establish strong spatial memories in chickadees (Baker et al., 1988). Despite this fact, on average, chickadees cache only 10 -15 % of the food they encounter in the wild in a day, suggesting that they do rely heavily on, and consume a large amount of, foraged food (Pravosudov, 1985).

1.8 Foraging in the Win-Shift/ Win-Stay Paradigm

Foraging animals can employ two strategies: win-stay to return to the location of food, or win-shift to avoid that location in the future (Olton, Handelmann & Walker, 1981). These strategies are cognitive adaptations to the depleting or replenishing nature of a food source (Sulikowski & Burke, 2011). Win-stay and win-shift strategies are typically tested in a two-phase procedure. In the first phase, some spatial locations are rewarding because they are baited with food. In the second phase, animals will win-shift when they avoid, rather than return to, recently rewarded or baited locations. Animals that return to recently rewarded locations are said to win-stay. Birds that forage on food resources that deplete slowly, such as seedheads, use a win-stay strategy (Kamil, 1978), and birds that forage on resources that deplete, such as nectar, use a win-shift strategy (Smith, 1974). Black-capped chickadees forage on foods that deplete rapidly, such as insects, and foods that deplete slowly, such as seedheads (Pravosudov, 1985), so it is hypothesized that they could use either strategy, depending on reward contingency. Rats tested on a radial maze flexibly employ both win-shift and win-stay strategies in response to reward contingencies (Guitar & Roberts, 2014). The Win-stay/Win-shift paradigm relies heavily on hippocampal functions such as spatial working and reference memory (Olton, Handlemann & Walker, 1981).

1.9 The Current Study

The present research investigated the role of decreased hippocampal neurogenesis on spatial working and reference memory, and foraging in a win-shift/win-stay task. In the first

experiment, two groups were tested in spatial working and reference memory tasks. The treatment group received methylazoxymethanol acetate, a neurotoxin that decreases hippocampal neurogenesis in chickadees. The reference memory task required subjects to remember six out of twelve locations in trees that consistently contained a food reward, and was followed by a reversal task. The working memory task required subjects to retrieve one food reward from twelve different locations and to keep track of which locations had already been searched within a trial during testing. It was hypothesized that if neurogenesis aids memory, MAM treated subjects would perform less well than controls. If neurogenesis disrupts memory, MAM treated subjects should perform better than controls, due to decreased interference by new neurons. The process that differentiates similar memories during encoding, pattern separation, may have been reduced by decreasing neurogenesis because new neurons could not be recruited into memory circuits. The results indicate that a reduction in adult hippocampal neurogenesis has no effect on the performance of a spatial working memory task or a well-learned spatial reference memory task. However, a nonsignificant difference between groups during the reversal suggests that hippocampal neurogenesis may contribute to successfully differentiating similar spatial memories at the time of encoding.

In the second experiment two groups of Black-capped chickadees were tested in a spatial memory task to determine their spontaneous foraging strategy and whether they could flexibly employ win-shift or win-stay strategies depending on reward contingencies. Chickadees searched for food rewards in Phase A, and after a short interval, searched again in Phase B, with reward contingencies in Phase B reinforcing either a win-shift or win-stay strategy. The number of searches they were allowed to make in Phase B varied across three motivational conditions. It was hypothesized that because Black-capped chickadees forage on foods that deplete slowly and rapidly, they would be able to Win-shift or Win-stay in response to reward contingency. Chickadees showed a win-shift strategy within each phase, but did not employ either a win-shift or win-stay strategy between phases, regardless of reinforcement. While chickadees successfully employ a win-shift strategy within a foraging bout, they appeared indifferent to the renewal and depletion properties of food sources over a longer time scale. It is possible that birds had individual preferences for particular locations. It is also possible that the paradigm is not a valid measure of spatial search across species, and that we should be cautious when applying sweeping general models to complex and dynamic behaviors.

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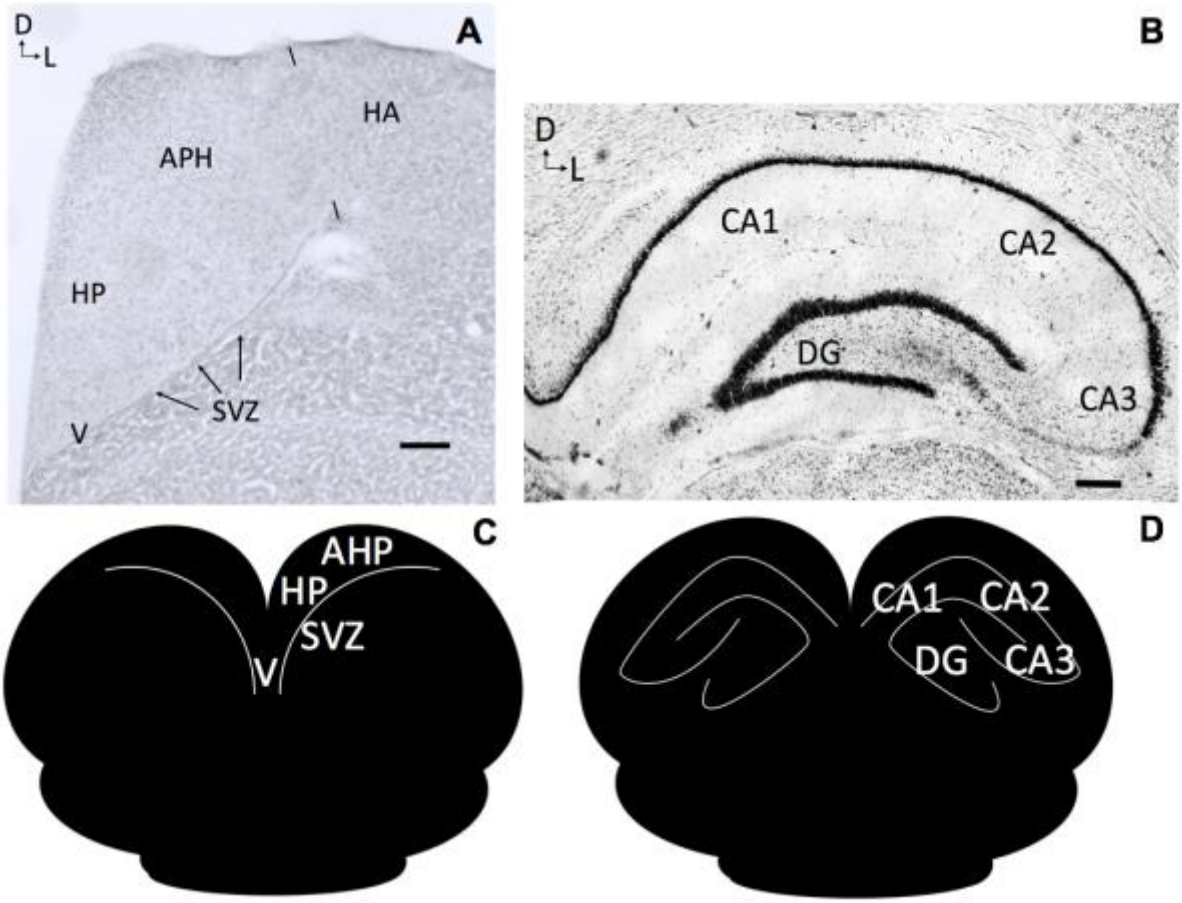

Figure 1.1 A) Black-capped chickadee hippocampus showing the hippocampus (HP) and the area parahippocampalis (APH). Solid lines mark the boundary with hyperpallium apicale (HA). The subventricular zone (SVZ), shown by three pointed arrows, is the region of stem cell division that produces both neurons and radial glial cells. The ventricle (V) is also indicated. Cells are labelled with Nissl stain (scale bar, 40 μm). (B) Major field divisions in the adult rat hippocampus. The dentate gyrus (DG), and three CA subfields (CA1, CA2 and CA3) are shown. Cells are labelled with Nissl stain (scale bar, 500 μm). D = dorsal, L = lateral. Drawings show the location of the hippocampus in coronal views of the chickadee (C) and rat (D) brains.

2.1 ADULT HIPPOCAMPAL NEUROGENESIS MAY AID PATTERN SEPARATION IN BLACK-CAPPED CHICKADEES BUT DOES NOT INFLUENCE SPATIAL WORKING OR REFERENCE MEMORY

Introduction

The ability to retrieve information in memory is essential for human and animal survival. Neurogenesis, the production of new functional neurons in the brain, may influence memory retrieval. However, the function of adult hippocampal neurogenesis is not well understood. It has been proposed to both aid in the formation of memory (Deng, Aimone & Gage, 2010), and to disrupt memory (Frankland, Köhler & Josselyn, 2014). For decades it was believed that neurogenesis did not occur into adulthood despite the early work of Das and Altman (1972). Later research conducted by Goldman and Nottebohm (1983) uncovered neuronal precursors in the song control nuclei of canaries. Adult neurogenesis has now been discovered in the hippocampi of mammals (Gross, 2000; Gould & Gross, 2002) and birds (Nottebohm, 1981; Goldman & Nottebohm, 1983; Barnea & Nottebohm, 1996) among other classes. In mammals, new neurons are generated in the subgranular zones and migrate to the dentate gyrus and CA subfields of the hippocampus and olfactory bulb (Kaslin et al., 2008). In the bird brain, new neurons are generated along the walls of the lateral ventricles and migrate radially throughout the brain extending beyond the hippocampus and olfactory areas (See Figure 1.1; Kaslin et al., 2008; Vellema et al., 2010).

It is currently unknown how changes in adult neurogenesis influence hippocampal functions such as spatial memory. Spatial memory for the locations of food, danger, and shelter is essential for survival and development. Spatial working memories are memories for the distinctive features of an experience, while spatial reference memories are memories for the stable features of an experience (Olton, Becker & Handelmann, 1979). Chickadees have impressive spatial memory and are able to keep track of thousands of stored food locations (Sherry, 1984; Healy & Hurly, 2004). Black-capped chickadees also experience adult hippocampal neurogenesis (Barnea & Nottebohm, 1994). Because of their specialized hippocampus, chickadees are an excellent model for the study of spatial memory and adult hippocampal neurogenesis. The chickadee hippocampus is the site of high levels of neuroproliferation (Hoshooley & Sherry, 2007) and chickadees have been used successfully as models for studying neurogenesis in the past (Barnea & Pravosudov, 2011; Hall, Delaney &

Sherry, 2014). Their brains also show a remarkable homology to brains of mammals, including homology of the hippocampal region (Jarvis et al., 2008). The current experiment will investigate how changes in adult hippocampal neurogenesis influence existing spatial working and reference memories.

In 1971 Marr wrote that little information about a single learnt event is required to provoke its recall, and suggested a mathematical model of a pre-existing structure responsible for the direct storage of memory associations. Today we know that memory retrieval relies on pattern completion: reactivation of the pattern of cellular activity that occurred during encoding (Frankland, Köhler & Josselyn, 2014), and that successfully distinguishing between two memories relies on pattern separation: differentiating the cellular activation associated with two memories by reducing the average overlap of brain activation between them (Treves & Rolls, 1994). Changing levels of neurogenesis influence both of these processes. The current study focused on the impact of decreasing cell proliferation in the hippocampus on spatial working and reference memory and pattern completion and separation processes. Neurogenesis can be manipulated experimentally with chickadees using methylazoxymethanol acetate (MAM), a neurotoxin that disrupts DNA synthesis and suppresses cell proliferation in the brain without significantly changing any measure of body composition (Hall et al., 2014). In rats, MAM has decreased neuroproliferation by 84% (Shors et al., 2001; 2002) and significantly altered hippocampal functions such as spatial memory.

Both working and reference memory have been linked to hippocampal functions (Clelland et al., 2009; Deng, Aimone & Gage, 2010; Scarf et al., 2014). Since neurogenesis decreases with age, and affects diseases and disorders of the nervous system that can affect memory, it is important to understand exactly what role neurogenesis plays in memory. I hypothesized that if neurogenesis aids memory, MAM treated subjects would perform less well than controls. If neurogenesis disrupts memory, MAM treated subjects would perform better than controls, due to decreased interference by new neurons.

2.2 Method

2.2.1 Birds

Twenty-four Black-capped chickadees, 11 female and 13 male, were captured by rectangular wire Potter traps between September 2015 and November 2016 near the Western University campus in London, Ontario, Canada. Birds were housed individually on a 10:14

light:dark cycle and provided with food and water *ad libitum* except during brief periods of food deprivation as described below. The light cycle was chosen for experimenter convenience, to mimic natural light cycles, and to ensure food deprivation as sufficient to result in motivation during testing. Food was powdered sunflower seeds mixed with powdered Mazuri Small Bird Diet (PMI Nutrition International, Brentwood MO). All animals were handled and tested according to the guidelines of the Canadian Council on Animal Care and protocols approved by the University of Western Ontario Animal Use Committee.

Studies using systemic MAM treatment to suppress neurogenesis have been criticized because MAM does not specifically target neurons but all cells with its antimitotic effects. Some researchers have argued that MAM-induced learning deficits may be associated with non-neuronal effects on cellular proliferation or general health of an animal (Dupret et al., 2005). To address these concerns, I monitored several components of body condition over the whole study using quantitative magnetic resonance scanning (QMR) including; fat mass, lean mass, free body water and total body water (g) to monitor nonspecific effects on birds health. Birds' body condition was measured the day preceding the first MAM or saline treatment, and following the last trial of testing.

2.2.2 Testing Apparatus

Before testing, birds were randomly assigned to either the MAM or control group (n = 12 per group). Birds were tested in four cohorts (n = 6 per cohort) in counterbalanced order (working memory group (n = 6), reference memory group (n = 6), working memory group (n = 6), and reference memory group (n = 6)). Sex, determined at sacrifice was not known at the time of group assignment. Previous studies have found no sex difference in food-caching behavior, memory for cache sites, or relative size of the hippocampus in Black-capped chickadees (Petersen & Sherry, 1996). Birds were tested in an indoor aviary measuring 2.74 m X 2.74 m with a one-way mirror to allow live behavioral scoring by an observer. Although a small physical size, room size is comparable to the area in which birds would engage in focused flock foraging. The aviary contained two tree branches that were supported vertically. Tree 1 was larger than Tree 2 and contained eight, spaced out holes. It had 14 branches with the highest 78 inches from the floor, and the lowest, 37 inches from the floor. Tree 2 contained four, spaced out holes (twelve holes in total) drilled into the branches and labeled with white marker. Tree 2 had 6 branches with the highest hole being 65 inches from the floor, and the lowest hole being 37

inches from the floor. Birds' home cages were attached to the wall of an adjoining holding room. A 7.5 inch X 7.5 inch door in each cage could be opened remotely to admit a bird to the testing aviary (see Figure 2.1).

2.2.3 Training and Testing

Birds were food deprived at least 2 h before entering the aviary and all training and testing procedures occurred between 10:00 am and 1:00 pm seven days a week until experiment completion. To ensure birds initially experienced finding food at all twelve locations, I baited all holes with a sunflower seed fragment (sieved through 3 mm mesh) and plugged all holes with a piece of knotted green yarn. Yarn was not used after habituation in training or testing to avoid visual cues of visited locations. Each bird was released individually for 10 min in the testing aviary to find seeds. At the end of a trial the lights were turned off and birds returned independently to their home cages. The birds first experienced seven habituation trials where food was found in all twelve locations covered by knotted yarn. Criterion for habituation was retrieving ten or more seeds within the first twelve searches for three days in a row. Twenty training trials were provided for the birds to learn either the working or reference memory task, followed by a twenty-day break before testing trials (See Appendix A.2.1 and A.2.2). Testing trials ranged from 32 to 39 days depending on the task.

2.2.3.1 Working memory task.

The working memory task contained 32 trials in which all twelve locations were baited with a seed fragment. Birds were required to complete a within trial working memory task and retrieve all twelve seed fragments. Revisits to holes previously visited within a trial were counted as working memory errors.

2.2.3.2 Reference memory task.

The reference memory task contained 35 trials for cohort two and 39 trials for cohort four in which only six pseudo-randomly assigned locations were baited with a seed fragment. Locations were chosen such that the proportion of baited locations on each tree was identical (four out of eight on Tree 1, and two out of four on Tree 2). The six holes varied among subjects but each set was always matched between a MAM and control subject. Birds were required to complete a between-trial reference memory task to retrieve all six seed fragments. Revisits to holes previously visited, or visits to unbaited holes within a trial were counted as memory errors.

All birds experienced a single reversal during trials 33-39 where the holes that now contained food switched from the bird's usual set of six, to the opposite six holes.

After retrieving a fragment from all baited holes, all birds remained in their home cages for the remainder of the day. Each bird entered the aviary and searched for food until all rewards were found or until 10 minutes elapsed. The order in which birds were tested was randomized daily to prevent systematic differences in the time an individual bird had been food-deprived before testing. All injections of saline or MAM occurred on trials 6-11. Kim et al. (1999) demonstrated that new neurons become anatomically integrated in the adult avian brain anywhere between 9 and 15 days following their production. Because it is difficult to relate the recruitment and anatomical integration of new neurons to changes in behavior, I continued training birds before, during and following MAM or saline treatment to ensure I would be able to capture effects on behavior up to 21 days following the initiation of treatment.

2.2.4 Behavioral Scoring

An observer blind to birds' treatment condition observed all trials from behind a 6-foot one-way mirror and recorded behavior. On the working memory test, retrieval accuracy was measured as the number of baited holes visited in the first twelve searches, where a score of twelve out of twelve, or zero errors made, indicates perfect performance. For the working memory task, revisits to a hole were scored as searches and the number of correct holes changed as search proceeds. The number of correct choices expected by chance corresponds to an "occupancy problem" (Feller, 1967), as for the classic radial arm maze (Olton & Samuelson 1976). For twelve holes, all twelve of which are initially correct, and twelve searches, including revisits, the number of correct choices expected by chance when sampling from a binomial distribution equals 7.76. The number of expected errors by chance equals 4.24.

On the reference memory task, retrieval accuracy was measured as the number of baited holes visited in the first six searches, where a score of six out of six, or zero errors made, indicates perfect performance. For the reference memory task, revisits were scored as searches and the number of correct choices expected by chance therefore corresponds to sampling with replacement from the binomial distribution. For twelve holes, six of which are correct, and six searches, including revisits, the number of correct searches expected by chance equals 3. The number of expected errors by chance is also 3. A search and revisit were defined as either eating

the seed fragment found inside the hole, or inserting the beak into the hole, or looking directly into a hole at a distance within ~2 cm.

2.2.5 Suppression of Neurogenesis

The lean mass, fat mass, total body water and free body water of each bird was measured using QMR body-composition analysis (Gerson & Guglielmo, 2011). Starting on trial six of testing in both the working and reference memory tasks, birds received a daily injection of either methylaoxymethanol acetate (MAM group; 14 mg/kg; i.m.) dissolved in 0.1*M* phosphate buffered saline (PBS; pH = 7.4), or PBS vehicle (control group, i.m.) following testing each day for six days. MAM is an antimitotic drug that reduces the number of adult-born neurons by causing DNA damage via the methylation of guanine residues (Matsumoto & Higa, 1966). MAM is commonly used to impair adult neurogenesis in rodents (Shors et al., 2001, 2002) and has been used effectively in chickadees (Hall et al., 2014).

Doublecortin (DCX) immunohistochemical labeling was used to determine the number of new neurons in the hippocampus. DCX is expressed in proliferating progenitor cells and newly generated neuroblasts (Brown et al., 2003). DCX labeling around the ventricular wall provided data on cell proliferation after six days of MAM (or saline) treatment. Additional weeks of behavioral testing followed MAM treatment in order to capture the effect of neurogenesis. I continued testing birds for twenty-one to twenty-nine days following the last MAM or saline injection to detect any impairment in spatial memory that might occur during reduced recruitment and incorporation of new neurons, as described for rodents (Snyder et al., 2005; Shors et al., 2012). DCX labeling was measured in five areas of hippocampus; the Subventricular Zone (SVZ), all area of hippocampus excluding the subventricular zone (NON-SVZ), the darkly staining V (V), the hippocampal cap (CAP), and the area parahippocampus (APH) as described by Atoji and Wild (2006). Six days of MAM treatment introduced a temporal pulse of impaired cellular proliferation expected to reduce the number of new neurons available for incorporation in the brain.

2.2.6 Tissue Collection and Processing

The day following the last trial of testing birds fat mass, lean mass, free body water, and total body water were recorded by QMR. Birds were then deeply anesthetized with isoflurane and transcardially perfused with phosphate buffered saline (PBS; pH 7.4) followed by 4% paraformaldehyde. Brains were dissected from the skull, submersed in 4% paraformaldehyde

overnight, cryoprotected in 30% sucrose in PBS for a minimum of 40 h, frozen on pulverized dry ice, and stored at -80°C until sectioning. Sex was determined at this time by examination of the gonads.

Brains were sectioned coronally (thickness = 40 µm). Once the subventricular zone (SVZ) was reached, as identified by whole-brain morphology, and every tenth section in three alternating series was collected, until no sections containing hippocampus were remaining in each brain (see Appendix B.2). Brains were stored at 4°C until histology, which occurred within 48 h of sectioning.

The main tissue series was stained to visualize DCX labeling. Specifically, tissue was washed twice in PBS (pH 7.5) for 5 min with agitation before being incubated in 30% H_2O_2 for 15 min, followed by two more rinses in PBS. The tissue was then incubated in 10% Normal Horse Serum (Vector Labratories) in 0.3% Triton X-100 (Sigma) for 1 h at room temperature with agitation. Tissue was then incubated in stock DCX (C-18) primary antibody 1:250 in in 0.3% Triton X-100 (Sigma) overnight at 4°C. The following day tissue was rinsed twice in 0.1% PBS/T for 5 min with agitation. Tissue was incubated in biotinylated secondary antibody Horse Anti-Goat IgG 1:400 with 0.3% PBS/T for 1 h at room temperature with agitation. Next, tissue was rinsed twice in 0.1% PBS/T for 5 min with agitation before incubation in ABC Elite avidin-biotin horseradish-peroxide complex (Vector Laboratories) 1:200 with 0.3% PBS/T for 1 h at room temperature with agitation. Tissue was rinsed twice in 0.1% PBS/T for 5 min with agitation before it was reacted with 0.04% diaminobenzidene solution (Sigma) for 90 s to visualize antibody-avidin-biotin complexes before being rinsed 5 times with PBS. Sections were mounted on Superfrost glass slides (VWR) and left to dry for 48 h. Slides were dehydrated in a series of graded alcohol concentrations, cleared in xylene and coverslipped.

2.2.7 DCX Quantification

I used StereoInvestigator software (version 10, Micro-brightfield, Colchester, VT) for all stereological measurements. I determined the boundaries of the hippocampal formation as described in Krebs et al. (1989), and Atoji and Wild (2006). I divided the hippocampus into subventricular (SVZ) and non-subventricular (NONSVZ) zones, and also into the darkly staining V region (V), the hippocampal cap (CAP) and the area parahippocampus (APH) (See Figure 2.2 and 2.3). I used a total of 5 sections per bird for hippocampal measurements (400 µm apart). I used a grid size of 280 µm for the NON-SVZ, CAP and APH regions, and a grid size of 180 µm

grid size for the SVZ and V regions (see Figure 2.4). Accurate grid sizes were determined through a pilot study. All sections were coded prior to the analyses; so all measurements were performed blind with respect to bird identity and experiment group.

To calculate the total number of hippocampal neurons I used the optical fractionator method (West et al., 1991), which combines the fractionator (multistage sampling scheme) with the optical dissector to allow for unbiased counting of neurons (Sousa et al., 1998). This method allows an estimation of the relative number of neurons between groups (West et al., 1991). In our analyses, I used a 60 µm counting frame (see Figure 2.5). I used a 40x objective on a Nikon 90i Optiphot microscope linked to the PC-based Stereo-Investigator system. To evaluate the precision of my sampling scheme, I calculated coefficients of error for neuron count measurements. I calculated the range of individual estimates, which allows evaluation of the robustness of our sampling scheme (CE; Schmitz & Hof, 2000; West et al., 1996). The variance of estimates was low (less than 10% error) for neuron counts in three regions (See Table 2.1). The variance of the hippocampal cap and darkly staining V regions was high, likely because of cell density differences.

2.2.8 Statistical Analysis

I compared DCX cell counts along the ventricle in the SVZ, the rest of the hippocampus NONSVZ, the V, the APH, and the CAP using an independent samples *t*-test with treatment (MAM vs. control) as the between-subjects factor.

To test for the effects of MAM on body condition, I compared lean mass, fat mass, total body water, and free body water before and after treatment with paired samples *t*-tests.

To test for the effects of MAM on reference and working memory performance I conducted two repeated measures ANOVAs with trial as a within-subject factor and group as the between-subjects factor. A separate repeated measured ANOVA was conducted for the reversal phase of the reference memory task. A Greenhouse-Geisser correction factor was used.

2.3 Results

2.3.1 Neurogenesis

I found that six daily injections with MAM significantly reduced hippocampal neuroproliferation in the brain of chickadees measured by DCX labeled cell counts in the non-subventricular zone (NONSVZ), $t(22) = 3.149$, $p < .01$, and the area parahippocampus (APH), $t(22) = 3.426$, $p < .01$ (see Figure 2.6). Hall et al. (2014) noted that their subjects experienced a

significant reduction in neuroproliferation by MAM of 46% using the same dosage of 14 mg/kg. I found a 38% reduction in neurogenesis in the non-subventricular zone (NONSVZ), and a 42% reduction in the area parahippocampus (APH).

2.3.2 Body Condition

Body condition was measured before and after MAM or saline administration by QMR and compared in a paired samples *t*-test (See Appendix C.2.1). No significant differences in neither control nor MAM subjects were found before or after MAM or saline administration (see Appendix C.2.2 – C.2.5).

2.3.3 Working Memory Retrieval Accuracy

There was no significant difference in the number of errors made between MAM and control subjects, $F(1, 10) = 1.12$, $p > .05$, $\eta_p^2 = 0.10$, and both groups performed significantly better than chance: MAM, $t(31) = 36.28$, $p < 0.001$; control, $t(31) = 35.88$, $p < 0.001$. There was no effect of trial, $F(7.01, 70.11) = 1.88$, $p > .05$, $\eta_p^2 = 0.16$, and no significant interaction between group and trial $F(7.01, 70.11) = 1.37$, $p > .05$, $\eta_p^2 = 0.12$ (see Figure 2.7).

2.3.4 Reference Memory Retrieval Accuracy

2.3.4.1 Trials 1-32 (N = 12). There was no significant difference in accuracy between MAM and control subjects, $F(1, 10) = 2.05$, $p > .05$, $\eta_p^2 = 0.17$, and both groups performed significantly better than chance: MAM, $t(31) = 29.20$, $p < 0.001$; control, $t(31) = 21.24$, $p < 0.001$. There was a significant effect of trial, $F(6.68, 66.78) = 3.85$, $p < .05$, $\eta_p^2 = 0.28$, such that fewer errors were made as the number of trials increased. There was no interaction between trial and group $F(6.68, 66.78) = 1.10$, $p > .05$, $\eta_p^2 = 0.10$ (see Figure 2.8).

2.3.4.2 Reversal trials 33-35 (N = 12). There was no significant difference in accuracy between MAM and control subjects, $F(1,10) = 0.31$, $p > .05$, $\eta_p^2 = 0.003$, and neither group's performance differed from chance: MAM, $t(2) = 1.14$, $p > 0.05$; control, $t(2) = 0.09$, $p > 0.05$ (see Figure 2.8).There was a significant effect of trial $F(1.80, 18.00) = 9.10$, $p < .05$, $\eta_p^2 = 0.48$, such that trial 33 (M = 4.50, SE = 0.22) differed significantly from 34 (M = 3.67, SE = 0.36) and 35 (M = 2.75, SE = 0.26) but 34 and 35 did not differ from each other. A significant interaction between trial and group was also found $F(1.80, 18.00) = 4.31$, $p < .05$, $\eta_p^2 = 0.30$. For the control group, trial 35 (M = 2.33, SE = 0.37) differed from trials 33 (M = 4.17, SE = 0.31) and 34 (M = 4.33, SE = 0.51). In the MAM treated group, trial 33 (M = 4.83, SE = 0.31) differed significantly from trials 34 (M = 3.00, SE = 0.51) and trial 35 (M = 3.17, SE = 0.37).

2.3.4.3 Reversal trials 33- 39 (N = 6). The second cohort was tested during the reversal for three more trials in order to investigate indications that MAM treated subjects were reversing more slowly than controls, and therefore trials 35-39 were tested with half as many subjects. There was no significant difference in accuracy between MAM and control subjects, $F(2.05, 9.76) = 2.05$, $p > .05$, $\eta_p^2 = 0.34$, and neither group's performance differed from chance: MAM, $t(6) = 1.96$, $p > 0.05$; control, $t(6) = 0.07$, $p > 0.05$ (see Figure 2.8). There was no significant difference in accuracy between MAM and control subjects, $F(1, 4) = 2.22$, $p > .05$, $\eta_p^2 = 0.36$. There was also no interaction between trial and group $F(2.05, 9.76) = 1.65$, $p > .05$, $\eta_p^2 = 0.29$.

2.4 Discussion

2.4.1 Suppressing Adult Neurogenesis

I found that six daily injections with MAM significantly reduced hippocampal neuroproliferation in the non-subventricular (NONSVZ) zone and area parahippocampus (APH) regions. Both of these regions had low coefficients of error in cell counts and therefore I am confident that MAM reduced neuroproliferation (see Table 2.1). I am not surprised that no differences in neuroproliferation were found in the darkly staining V (V), and the subventricular zones (SVZ) because perfusions occurred three weeks after the last treatment day and neuroproliferation in these regions is likely to have returned to normal levels in that three week period. New cells born at the time of last injection would have migrated away from the ventricle into the hippocampus. I would expect changes from our manipulation to be detectable in the hippocampus away from the neuroproliferative zone. Barnea and Nottebohm (1994) hypothesized a non-random distribution of new neurons and found that six weeks after birth, 95% of new neurons were found in a narrow band 350 μm away from the ventricular zone. No differences were found in the hippocampal cap (CAP) region likely because of cell density changes in this area and the large coefficient of error found while sampling cell counts there. For example, cells in the CAP region are sparse and clustered, leading to unreliable sampling counts.

2.4.2 Working and Reference Memory Task Retrieval Accuracy

I hypothesized that if neurogenesis aids memory, MAM treated subjects would perform less well than controls. If neurogenesis disrupts memory, MAM treated subjects would perform better than controls, due to decreased interference by new neurons. There are multiple explanations for why I found no difference in working or reference memory performance

between MAM treated subjects and controls. One possible explanation is that decreased neurogenesis does not influence a learned retrograde spatial working or reference memory. According to Frankland et al., (2014) a decrease in neurogenesis should cause less forgetting and increased pattern completion of retrograde memories. I found no such effects, but possible differences between subjects during the reversal are informative for future study. There is also evidence from some rodent studies showing that reduced neurogenesis fails to affect performance on the Morris water maze (Shors et al., 2001, 2002; Snyder et al., 2005). There is contradictory evidence in rodents suggesting that spatial ability is impaired in place recognition tests (Rola et al., 2004). It is clear that past results in this area have not been conclusive.

While decreasing neurogenesis protects existing memories, acquisition of new information that conflicts with previously stored information will be impeded in adult animals if neurogenesis is reduced after original learning as in the current study's reference memory reversal. There is some indication in Figure 2.8 that MAM treated birds were slower to show reversal in trials 33-39 but this difference was not significant. Evidence with rats demonstrates that reducing hippocampal neurogenesis typically results in impaired anterograde memory formation (Deng, 2009). In humans, evidence shows that reducing hippocampal neurogenesis prevents new hippocampal memory formation using the trace eyeblink-condition paradigm (Shors et al., 2001). These results fit with the prediction of Wiskott, Rash and Kempermann (2006), and Frankland et al., (2014), that new neurons are necessary to avoid catastrophic interference when new information is being learned. Hunsaker and Kesner's (2013) Attribute Model would also support this hypothesis by showing a failure of pattern separation during encoding of conflicting information. Increasing the length of the reversal and using multiple reversals would be useful to examine in more detail the effects of reduced neurogenesis on the acquisition of conflicting information.

Some theories provide alternate explanations for the given results. It is possible that no changes in performance were observed because committed neurons in an existing memory circuit have a survival advantage when neurogenesis levels change (Leuner et al., 2004). If neuronal changes are occurring near established circuits, they coexist with, rather than alter, established synaptic connections (Frankland et al., 2014). Other researchers argue that neurogenesis modulates pattern separation and the ability to distinguish between memories. When neurogenesis is inhibited, pattern separation suffers (Frankland et al., 2014). As described by

Frankland et al., (2014) the effect of reduced neurogenesis may be dependent on a number of factors such as: age at the time of neurogenesis reduction (Martinez-Canabal, 2012); the number of neurons targeted (Ko et al., 2009); the maturational stage of the targeted neurons at the time of learning (Gu et al., 2012); the type of behavioural task used to assess learning and memory (Shors et al., 2002). Reduced neurogenesis could hinder hippocampal functions (Deng, 2009), or facilitate pattern completion and memory retrieval (Frankland et al., 2014). When neurogenesis levels decline, like they do in Alzheimer's disease, pattern separation fails and it becomes difficult to distinguish between similar memories (Zhao, Deng & Gage, 2008).

I found that a reduction in adult hippocampal neurogenesis had little effect on spatial working or reference memory in chickadees searching for sites baited with food. Hippocampal neurogenesis may contribute to successfully differentiating similar spatial memories at the time of encoding, but this was not examined directly in this experiment. Understanding the role of neurogenesis in memory is invaluable. This research has implications for deciphering the role of neurogenesis in the hippocampus, a brain region susceptible to age-related changes, decreases in neurogenesis and pathological aging in humans (Wesnes, 2010; Rosenzweig & Barnes, 2003; Small et al., 2004). Since neurogenesis decreases with age, and impacts diseases and disorders of the nervous system that can affect memory, it is important to understand exactly what role neurogenesis plays in memory. This research could lead to studies of what types of behavioral or contextual therapies could reduce possible interference in memory caused by changing levels of neurogenesis.

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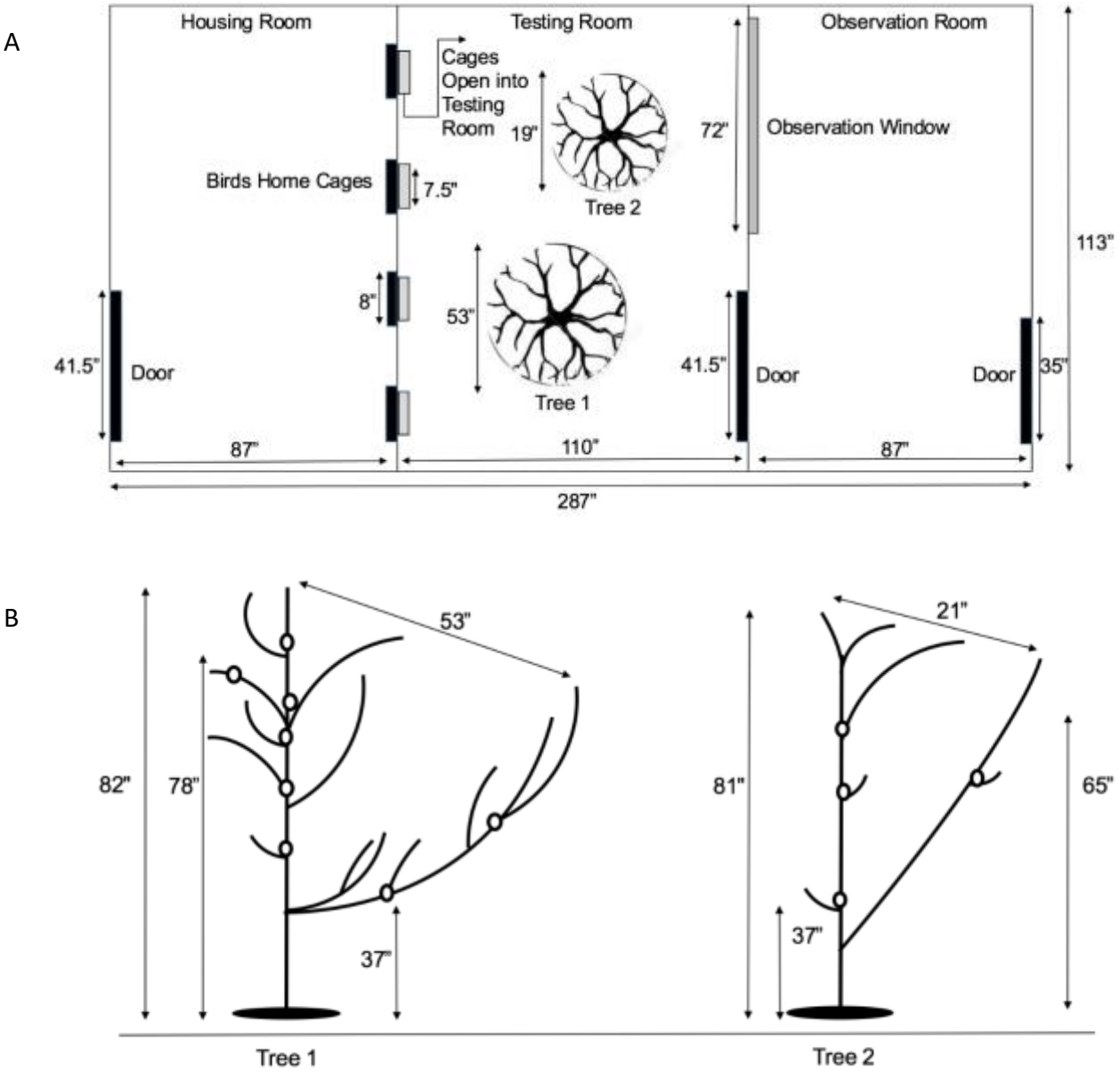

Figure 2.1. A) Scaled housing, testing and observation room figure with trees. B) Tree 1 and 2. Round white circles indicate the locations in which food rewards can be found. Various measurements are provided including; the distance from the highest point of the tree to the ground, the highest hole to the ground, the lowest hole to the ground and the width of the trees highest branch to the end of the furthest branch.

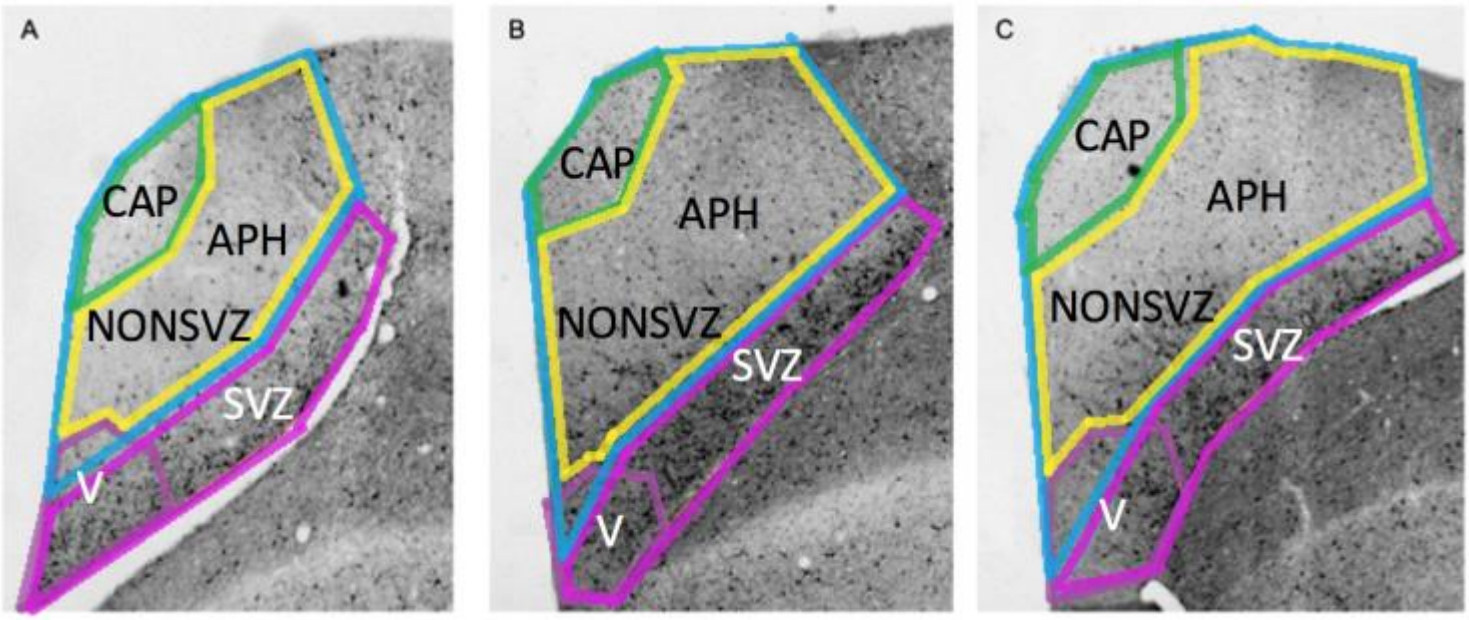

Figure 2.2. Rostral (A), Medial (B), and Caudal (C) sections of chickadee hippocampus with the contours for the five areas counted indicated. All five regions; the V (purple), APH (yellow), CAP (green), SVZ (pink), and NONSVZ (blue) are visible.

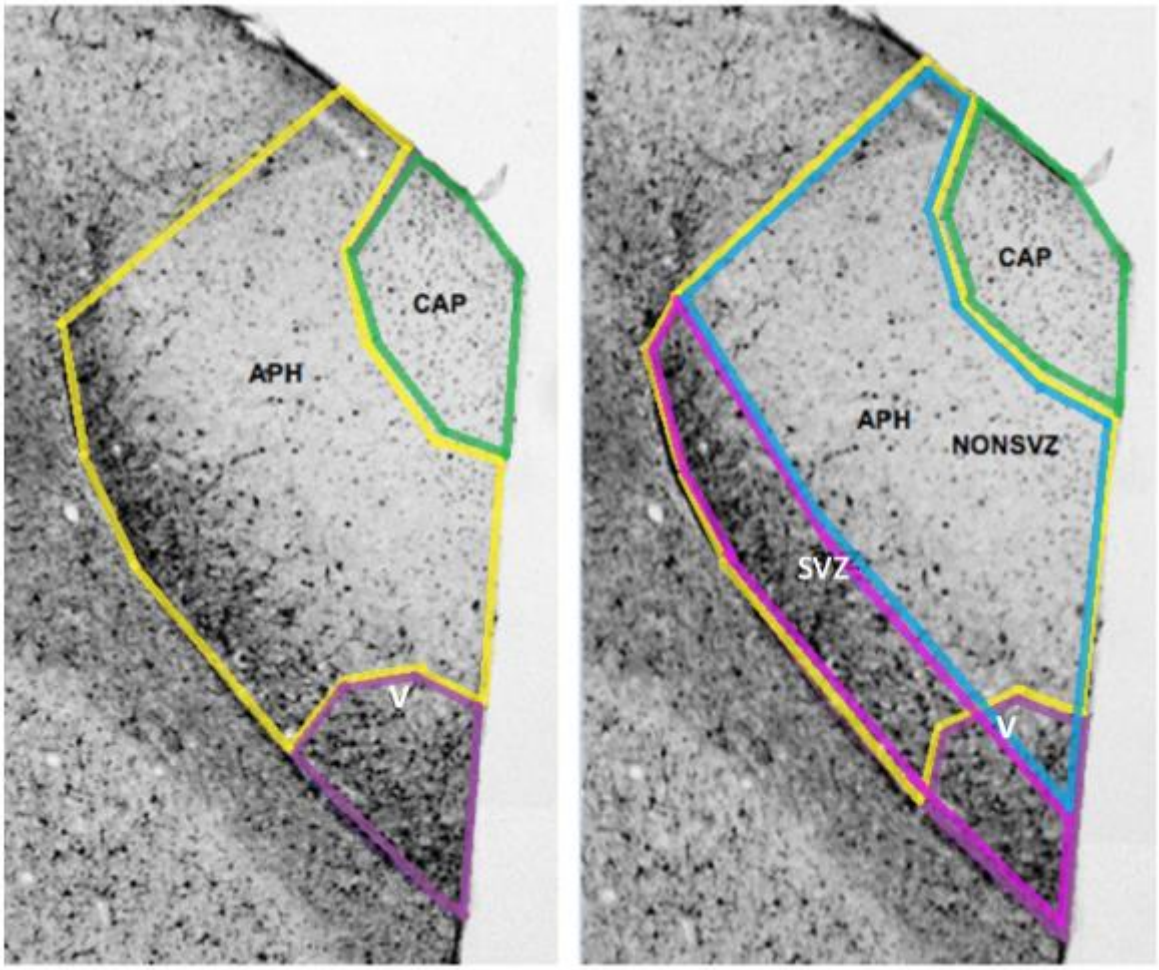

Figure 2.3. The contours used to define our five regions of interest within the hippocampus. On the left, the V, APH, and CAP contours are visible. On the right, all five regions; the V (purple), APH (yellow), CAP (green), SVZ (pink), and NONSVZ (blue) are visible. Note that the V and SVZ overlap, the V and the APH overlap, the V and the NONSVZ overlap, the SVZ and APH overlap, The NONSVZ, and APH overlap, and the NONSVZ and the CAP overlap. All regions were given independent cell counts.

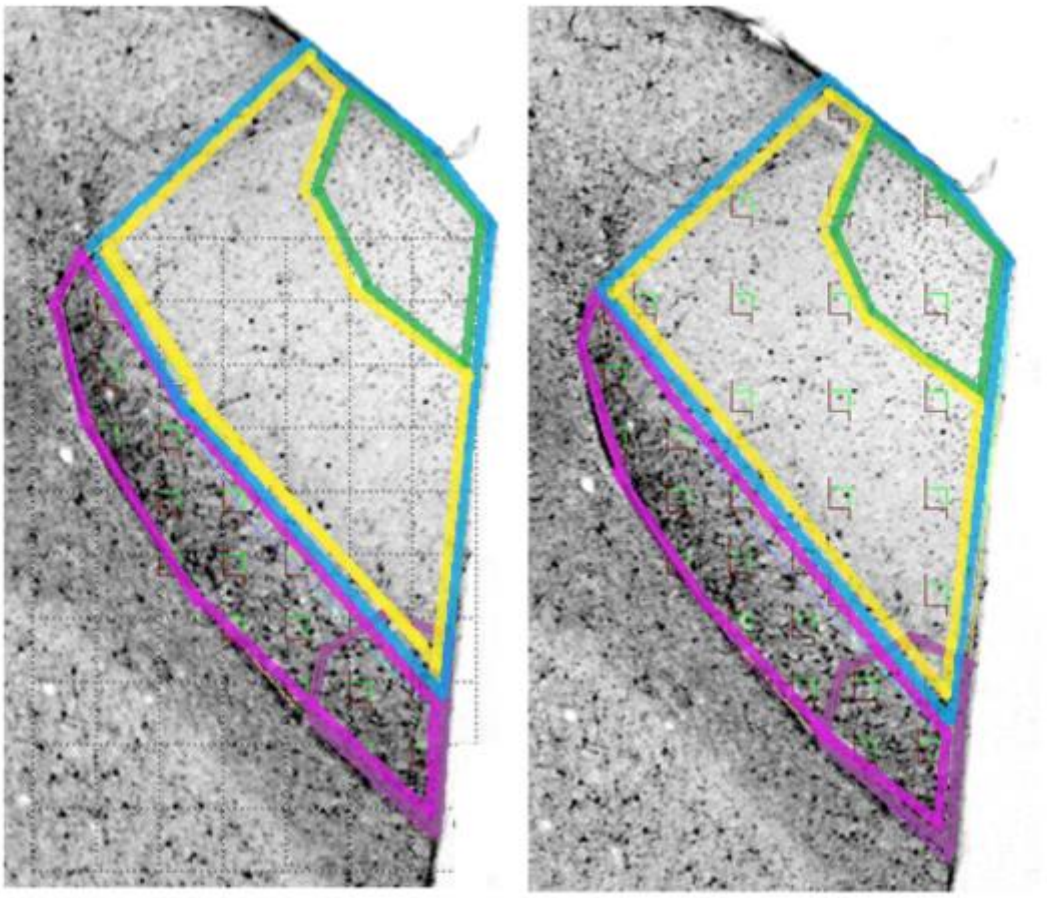

Figure 2.4. The grid layout for the subventircular zone (left) with the 180 µm counting frame grid visible, and the layout for the non-subventricular zone (right), with the 280 µm counting frame grid size visible. The left image shows the dotted-line grid created by Stereoinvestigator, while the right image excludes this feature.

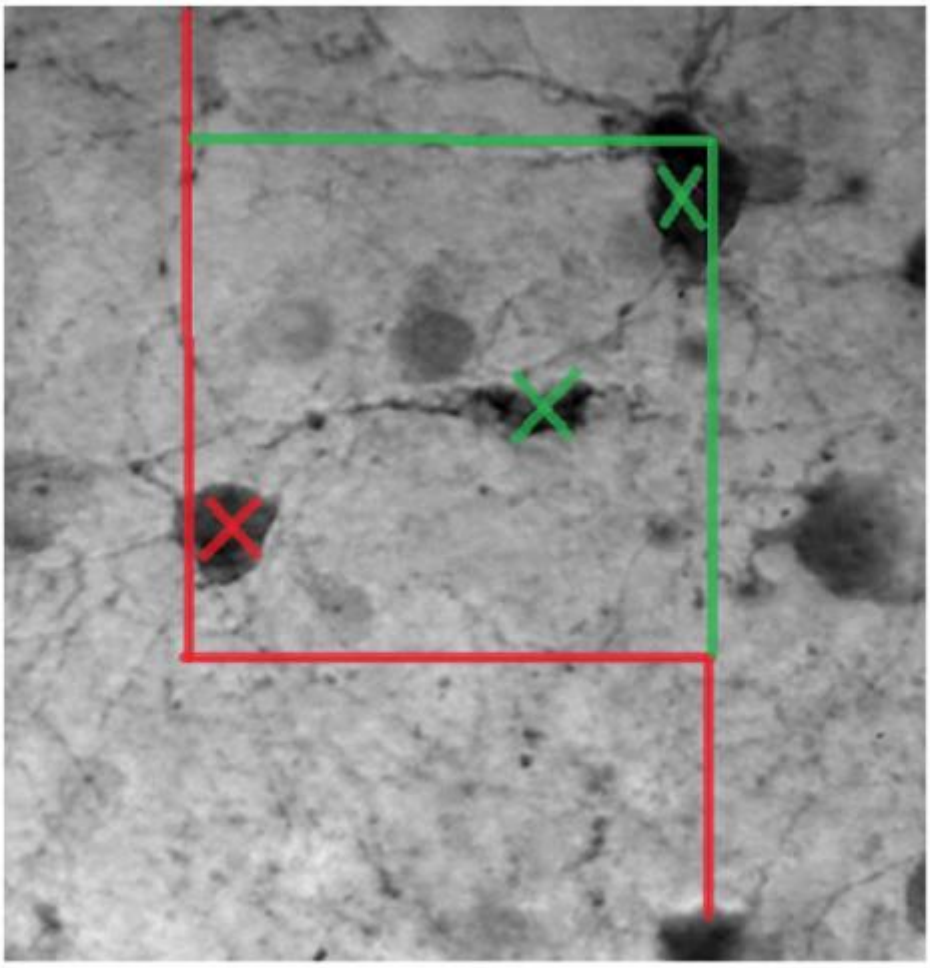

Figure 2.5. The counting frame. Only DCX lablled hippocampal cells completely within this frame or that intersect the green line at the top and right of the frame, are counted. Cells intersecting the red line at the bottom or left side of the frame are excluded from cell counts. The two labelled cells marked by a green X would be counted; the cell marked by the red X would not.

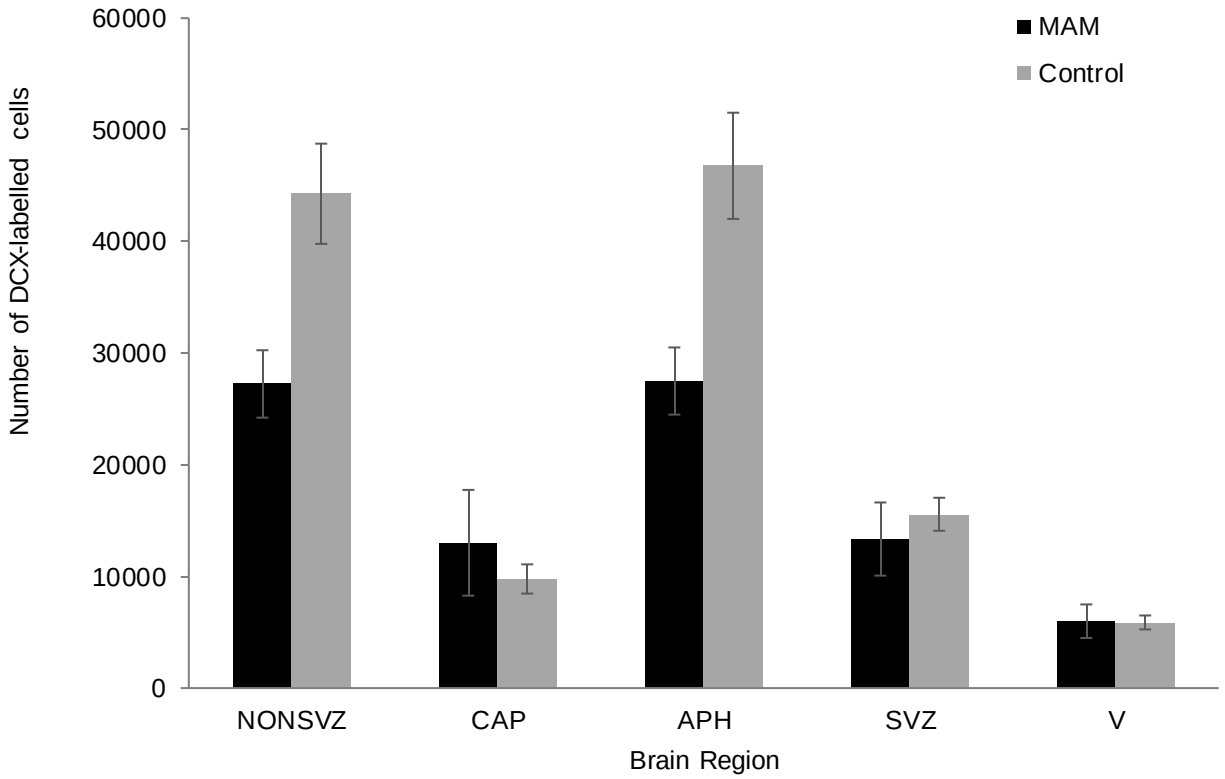

Figure 2.6. Cell counts for all five regions. In the NONSVZ and APH regions, control and MAM birds differed significantly in the number of DCX-labelled cells. Error bars represent the standard errors of the mean.

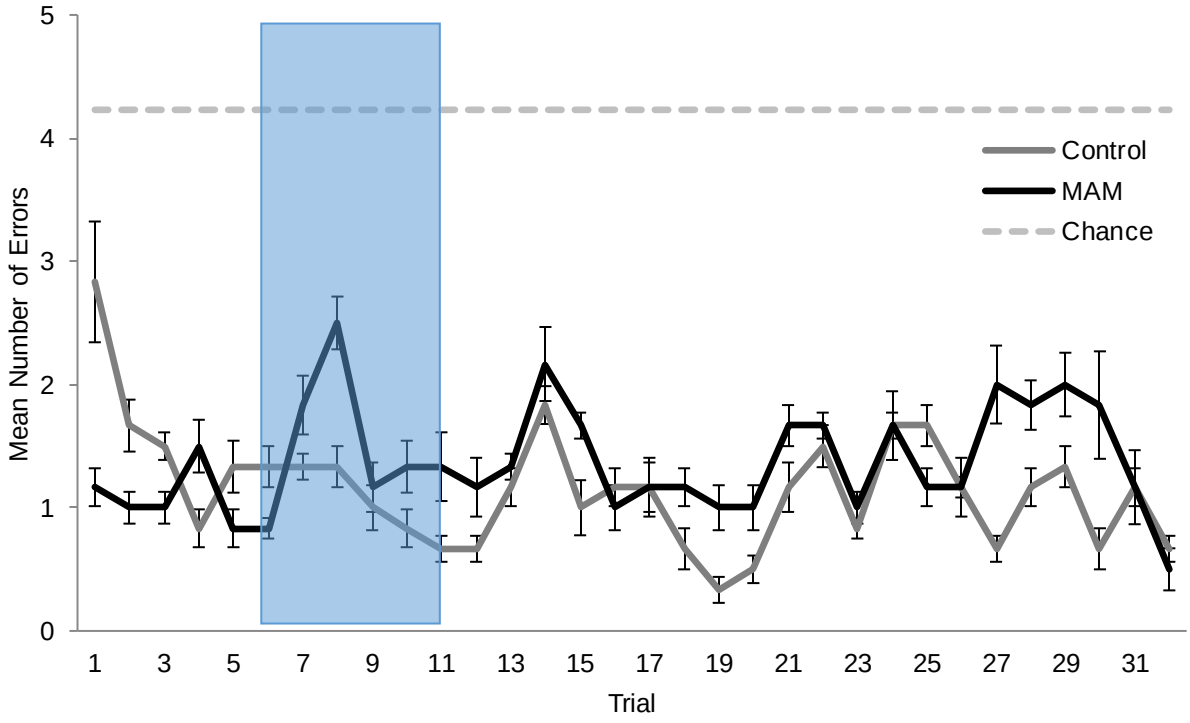

Figure 2.7. Mean number of working memory errors in the working memory task for MAM treated subjects and controls. MAM treatment occurred between trials 6-11. A reduction in the number of new neurons in the hippocampus would be expected from approximately trial 21 onwards. Error bars show standard errors of the mean.

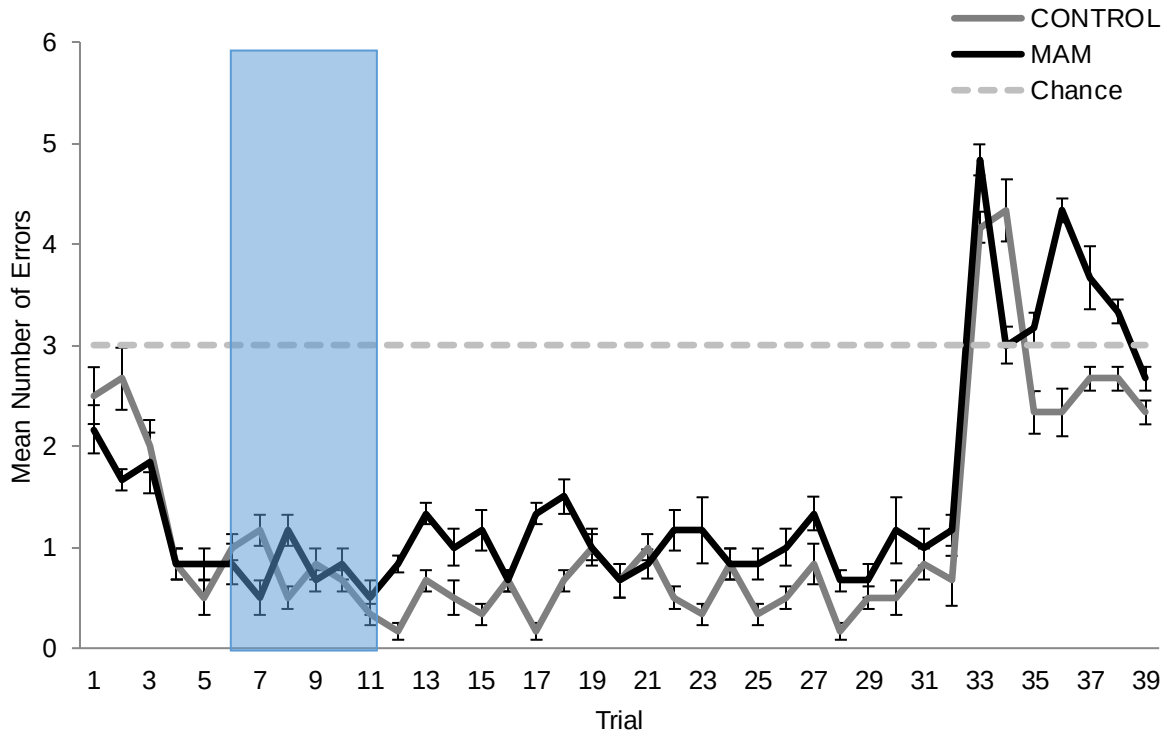

Figure 2.8. The mean number of reference memory errors in the reference memory task for MAM treated subjects and controls. MAM treatment occurred between trials 6-11. A reduction in the number of new neurons in the hippocampus would be expected from approximately trial 21 onwards. Reversal occurred at trial 32. All twelve subjects in each group completed trials 32-35. Six subjects from each group completed trials 35-39. Error bars reresent standard errors of the mean.

Table 2.1

Schmitz-Hof coefficients of error for each hippocampal region counted

Area	Mean CE	Range
Non-Subventricular Zone (NONSVZ)	0.07	0.07 – 0.09
Hippocampal Cap (CAP)	0.15	0.11 – 0.22
Area Parahippocampus (APH)	0.07	0.05 – 0.09
Subventricular Zone (SVZ)	0.08	0.05 – 0.09
Darkly Staining V (V)	0.12	0.09 – 0.17

Note. Hippocampal regions as described by Atoji and Wild (2006).

3.1 BLACK-CAPPED CHICKADEES DO NOT FLEXIBLY EMPLOY WIN-SHIFT OR WIN-STAY STRATEGIES IN A SPATIAL MEMORY TASK

Introduction

Foraging animals can employ two strategies: win-stay to return to the location of food, or win-shift to avoid that location in the future (Olton, Handelmann & Walker, 1981). These strategies are cognitive adaptations to the depleting or replenishing nature of a food source (Sulikowski & Burke, 2011). Win-stay and win-shift strategies are typically tested in a two-phase procedure. In Phase A, the study phase, subjects are presented with a number of locations they can search for food. Subjects are allowed to search until they retrieve all of the food rewards. After a delay, the subject returns for Phase B to search for food. When the baited locations are the same in both phases, subjects can employ a win-stay strategy to return to the locations of food. When the baited locations differ between phases, subjects can employ a win-shift strategy to avoid locations where food will not be found in the second phase. Rats tested on a radial maze flexibly employ win-shift and win-stay strategies in response to reward contingencies (Guitar & Roberts, 2014). Birds that forage on food resources that deplete slowly use a win-stay strategy (Kamil, 1978) while birds that forage on resources that deplete quickly use a win-shift strategy (Smith, 1974). Win-stay and win-shift behavior is hippocampus-dependent (Olton, Handlemann & Walker, 1981). Spatial memory for the locations of food, danger, and shelter is essential for survival and development and is well studied in both rodents and birds (Schmid Hempel, 2011). However, the responses of many birds to win-stay and win-shift contingencies is unknown. Black-capped chickadees are food-storing passerines that return to the locations of cached food (Sherry, 1984; 1989). They are able to keep track of thousands of stored food locations (Sherry, 1984; 1989), and their brains show a remarkable homology to the hippocampal region in mammals (Jarvis et al., 2008). Chickadees forage on resources that deplete after a single prey capture, such as insects, and foods that do not, such as seedheads. Chickadees would be expected to flexibly employ win-shift and win-stay strategies while foraging in response to reward contingencies. We tested chickadees under conditions that promoted either win-stay or win-shift strategies and quantified their use of each strategy. We hypothesized that chickadees would alter their foraging strategy depending on the reward contingency.

3.2 Method

3.2.1 Birds

Fourteen Black-capped chickadees were captured by Potter trap between September 2015 and November 2016 near the Western University campus in London, Ontario, Canada. Birds were housed individually on a 12:12 light:dark cycle and provided with food and water *ad libitum* except during brief periods of food deprivation as described below. The light cycle was chosen for experimenter convenience, to mimic natural light cycles, and to ensure food deprivation as sufficient to result in motivation during testing. Food was powdered sunflower seeds mixed with powdered Mazuri Small Bird Diet (PMI Nutrition International, Brentwood MO). All animals were handled and tested according to the guidelines of the Canadian Council on Animal Care and protocols approved by the University of Western Ontario Animal Committee.

Birds were divided into two groups: the Win-shift group (n = 7), and the Win-stay group (n = 7). The Win-shift group was tested two months prior to the Win-stay group, in a similar testing room with nearly identical trees and hole locations. Previous studies have found no sex difference in spatial memory including; food-caching behavior, memory for cache sites, and relative size of the hippocampus (Petersen & Sherry, 1996) and therefore sex was not determined in these subjects.

3.2.2 Testing Apparatus

Birds were tested in an indoor aviary measuring 2.74 m X 2.74 m with a one-way mirror to allow live behavioral scoring by an observer. Although a small physical size, room size is comparable to the area in which birds would engage in focused flock foraging. The aviary contained two tree branches that were supported vertically. Tree 1 was larger than Tree 2 and contained eight, spaced out holes. It had 14 branches with the highest 78 inches from the floor, and the lowest, 37 inches from the floor. Tree 2 contained four, spaced out holes (twelve holes in total) drilled into the branches and labeled with white marker. Tree 2 had 6 branches with the highest hole being 65 inches from the floor, and the lowest hole being 37 inches from the floor. Birds' home cages were attached to the wall of an adjoining holding room. A 7.5 inch X 7.5 inch door in each cage could be opened remotely to admit a bird to the testing aviary (see Figure 2.1).

3.2.3 Training and Testing

Birds were food deprived at least 2 h before entering the aviary and all training and testing procedures occurred between 10:00 am and 1:00 pm seven days a week until experiment completion. To ensure birds initially experienced finding food at all twelve locations, we baited

all holes with a sunflower seed fragment (sieved through 3 mm mesh) and plugged all holes with a piece of knotted green yarn. Each bird was released into the aviary individually for 10 min to find seeds. The birds were given seven of these habituation trials.

During testing chickadees entered the testing room individually through cage doors controlled by the experimenter. Chickadees searched the locations on the two trees freely for the duration of the trial and then returned home. All experiments consisted of two phases: Phase A, and Phase B, separated by 2-5 min. Between phases, the lights in the testing room were turned off and birds returned independently to their home cages. In each phase, half of the possible locations (6 out of 12) contained a seed fragment. The order in which birds were tested was randomized daily to prevent systematic differences in the time an individual bird was food-deprived before testing. Baited locations were pseudo-randomly selected for each trial. These locations remained the same between Phases A and B for birds in the Win-stay condition, and were different between phases for birds in the Win-shift condition. Locations were chosen such that half the locations on each tree were baited: four out of eight on Tree 1, and two out of four on Tree 2.

3.2.3.1 Experiment 1.

Experiment 1 consisted of eighteen trials. Every trial contained two phases. In Phase A birds searched until they found the six pseudo-randomly determined baited locations, or until 10 min elapsed. In Phase B birds searched until they found six baited locations, which were either the same locations as in Phase A (for the Win-stay group), or the six locations not baited in Phase A (for the Win-shift group).

3.2.3.2 Experiment 2.

Experiment 2 consisted of ten trials. Every trial contained two phases. In Phase A birds searched until they found the six pseudo-randomly determined baited locations, or until 10 min elapsed. In Phase B birds were allowed to search only six locations, regardless of whether or not they contained a reward. The purpose of restricting the number of locations birds were allowed to search was to increase the bird's motivation to choose correctly by imposing a limited number of searches as a cost. Again, the locations containing a food reward in Phase B were either the same locations as in Phase A (for the Win-stay group), or the six locations not baited in Phase A (for the Win-shift group).

3.2.3.3 Experiment 3.

Experiment 3 consisted of seven trials. Every trial contained two phases. In Phase A all twelve locations were baited. Birds were allowed to search six locations and consume the seed fragments before the lights were turned off and they were sent back to their home cage. In Phase B birds were permitted to make only six choices as in Experiment 2. The correct locations to find a food reward in Phase B were the same locations as those they chose in Phase A (for the Win-stay group), or the six locations not baited in Phase A (for the Win-shift group).

3.2.4 Behavioral Scoring

A search or a revisit were defined as either eating the seed fragment found inside the hole, removing the yarn and inserting the beak fully into the hole, or looking directly into a hole at a distance within ~ 2 cm. The measure of performance accuracy in Phase B – the number of correct searches – was the number of locations where food was found. Revisits were counted as an incorrect search or memory error.

3.2.5 Statistical Analyses

To test performance on each experiment of the win-shift and win-stay tasks I conducted a repeated measures ANOVA with trial as a within-subject factor and group (shift or stay) as the between-subjects factor. For all experiments, I determined the number of correct searches expected by chance from the binomial distribution assuming 12 locations, 6 of which were correct, and 6 search attempts, sampling with replacement. The expected number correct was 3 for both Win-shift and Win-stay conditions across all experiments. I also conducted multiple Kendall correlations to analyze the relationship between choices in Phase A and Phase B across trials for each experiment.

3.3 Results

3.3.1 Experiment 1

Neither the Win-shift, $t(17) = 1.57$, $p > .05$, nor the Win-stay, $t(17) = 0.083$, $p > .05$, group was more accurate than expected by chance in Phase B. There was no significant difference in accuracy in Phase B between the Win-shift and Win-stay groups, $F(1,12) = 0.003$, $p > .05$, $\eta_p^2 = 0.00$, no significant effect of trial, $F(6.23,74.81) = 1.68$, $p > .05$, $\eta_p^2 = .12$, and no significant interaction between trial and group $F(6.23,74.81) = 1.83$, $p > .05$, $\eta_p^2 = .13$ (see Figure 3.1).

Neither the Win-shift, $t(17) = 0.00$, $p > .05$, nor the Win-stay, $t(17) = 0.81$, $p > .05$, group was more accurate than expected by chance in Phase A. There was no significant difference in

accuracy in Phase A between the Win-shift and Win-stay groups, $F(1,12) = 0.54$, $p > .05$, $\eta_p^2 = .04$, no significant effect of trial, $F(6.87,82.45) = 2.05$, $p > .05$, $\eta_p^2 = .15$, and no significant interaction between trial and group $F(6.87,82.45) = 1.86$, $p > .05$, $\eta_p^2 = .14$ (see Figure 3.2).

3.3.2 Experiment 2

Neither the Win-shift, $t(9) = 0.08$, $p > 0.05$, nor Win-stay, $t(9) = 0.14$, $p > 0.05$, group was more accurate than expected by chance in Phase B. There was no significant difference in accuracy in Phase B between the Win-shift and Win-stay groups, $F(1,12) = 0.029$, $p > .05$, $\eta_p^2 = .003$ no significant effect of trial, $F(4.94,59.28) = 1.72$, $p > .05$, $\eta_p^2 = .13$, and no significant interaction between trial and group $F(4.94,59.28) = 2.16$, $p > .05$, $\eta_p^2 = .15$ (see Figure 3.3).

Neither the Win-shift, $t(9) = 0.113$, $p > 0.05$, nor Win-stay, $t(9) = 0.327$ $p > 0.05$, group was more accurate than expected by chance in Phase A. There was no significant difference in accuracy in Phase A between the Win-shift and Win-stay groups, $F(1,12) = 0.008$, $p > .05$, $\eta_p^2 = .05$, no significant effect of trial, $F(5.48,65.86) = 1.49$, $p > .05$, $\eta_p^2 = .11$, and no significant interaction between trial and group $F(5.48,65.86) = 1.19$, $p > .05$, $\eta_p^2 = .09$ (see Figure 3.4).

3.3.3 Experiment 3

Both the Win-shift, $t(6) = 4.70$, $p < 0.01$, and Win-stay, $t(6) = 10.68$, $p < 0.001$, groups performed significantly different from chance in Phase B. There was a significant difference between the Win-shift and Win-stay groups in Phase B, $F(1,12) = 22.49$, $p < .001$, $\eta_p^2 = .65$, no significant main effect of trial $F(4.42,53.05)$, $p > .05$, $\eta_p^2 = .04$, and no interaction between group and trial, $F(1.09, 53.05)$, $p > .05$, $\eta_p^2 = .08$ (see Figure 3.5). Birds chose which holes to visit in Phase A of this Experiment. Every hole visit in Phase A was baited, and therefore performance was not graphed for Phase A. For the Win-stay group, choosing their preferred locations repeatedly resulted in a larger number of correct searches than observed in Experiments 1 and 2. For birds in the Win-shift group, the locations they preferred in Phase A were never the correct holes in Phase B, resulting in lower numbers of correct searches than observed in Experiments 1 and 2. Regardless of reward contingency, birds appeared to have preferred locations they did visit and often these were holes located close together. A sequence analysis found many significant transitions between holes that were spatially close in proximity (see Figure 3.6). The significant difference between Win-stay and Win-shift birds likely occurred because Win-stay birds were rewarded for continuing to visit their preferred set of holes trial after trial, whereas the

Win-shift birds were not. Their high performance is confounded by their preference of holes across trials.

Win-stay bird's choices in Phase A and B across all three experiments were correlated more for birds in the Win-stay condition than in the Win-shift condition. While I expected Win-stay subjects' choices in Phase A and B to be highly correlated, since the correct locations are not changing between phases, I did not expect the same holes to be chosen across trials in Experiment 3. Interestingly, choices in Phase A and B correlated but were not dependent on reward contingency. Incorrect choices were made in Phase A, and then made again in Phase B. Because Experiment 3 was the only Experiment in which subjects chose their own set of correct holes, those in the stay condition were successful across phases and trials since they chose the same set of holes each time. Due to concerns that Win-stay birds were simply choosing preferred locations across trials I conducted a correlational analysis across Phases A and B of trials for each experiment (see Figure 3.7). All of the positive correlations, excluding Win-shift birds in Experiment 2, differed significantly from chance. None of the negative correlations differed significantly from chance (see Table 3.1). There were significant differences between the number of positive correlations between Phase A and B of trials between birds in the Win-Shift and Win-Stay groups, $F(1,12) = 8.72$, $p < .05$, $\eta_p^2 = .42$, such that birds in the Win-stay condition made choices in Phase B that positively correlated with their choices in Phase A significantly more than Win-Stay birds. There was no significant difference in the amount of significantly correlated trials across experiments, $F(1.22,14.65) = 3.91$, $p > .05$, $\eta p^2 = .25$, and no interaction between group and experiment, $F(1.22,14.65) = 0.18$, $p > .05$, $\eta_p^2 = .02$. There was no significant difference between the number of negative correlations between Phase A and B of trials between birds in the Win-Shift and Win-Stay groups, $F(1,12) = 0.54$, $p > .05$, $\eta_p^2 = .04$, no significant differences in the amount of significantly correlated trials across experiments, $F(1.44,17.31) = 3.32$, $p > .05$, $\eta_p^2 = .22$, and no interaction between group and experiment, $F(1.44,17.31) = 0.77$, $p > .05$, $\eta_p^2 = .06$.

3.4 Discussion

Chickadees spontaneously employed a win-shift strategy within each phase throughout all experiments, but failed to use either a win-stay or win-shift strategy between phases of the experiment. Chickadees performance in Phase B of Experiments 1 and 2 did not differ from chance, indicating that birds did not adopt the appropriate foraging strategy. In Experiment 2,

Win-stay birds showed a greater number of Kendell's Tau correlations between phases than Win-shift birds, suggesting that they were employing the correct strategy, Win-stay, to a greater extent than the Win-shift birds. In Experiment 3, where chickadees selected their own baited locations in Phase A, birds were biased towards choosing them again in Phase B, which resulted in above chance performance for the Win-stay group and below chance performance for the Win-shift group.

There are multiple explanations for the present results. Chickadee search strategies could be insensitive to changes in reward contingency as manipulated in our experiments, even though they forage on foods likely to promote both strategies. It is possible that the limited cost of using a random search strategy in our paradigm resulted in insufficient incentive for chickadees to employ more effortful foraging strategies. Since prey can influence an animal's foraging strategy (Sulikowski & Burke, 2015), chickadees may have failed to respond to reward contingencies because the same food reward was used throughout the experiment. Their choice of foraging strategy in the wild is not determined by reward contingencies, but by the resource they are collecting (i.e. replenishing or non-replenishing).

It may also be the case that because chickadees are a food-caching species they are less sensitive to the locations of found food. Chickadee memory for the locations of cached food is greater than that of the locations of found food (Baker at al., 1988). In fact, watching another bird store seeds does not help the watcher find those seeds, even though the same watcher has no problem recovering their own cached food. The perceptual and motor experience of finding food, carrying it to a location, and storing it there, may be necessary to establish strong spatial memories in chickadees (Baker et al., 1988). Despite this fact, chickadees cache only 10 -15 % of the food they encounter in the wild in a day, suggesting that they do rely heavily on foraged food (Pravosudov, 1985).

It may also be the case that chickadees location preferences have stronger influence on their foraging behavior than reward contingencies. The room may not have been large enough, and the consequence of searching nearby holes not costly enough, to discourage subjects from using preferential search patterns or relying on a motor memory. For example, a bird may search a particular hole, and then check the closest holes regardless of whether or not they are reinforced for doing so because the cost would only be a small delay in receiving food after the task. Optimal foraging models are based on the idea that an animal collects food in a manner that

will maximize its rate of energy intake (Pyke et al., 1977; Krebs et al., 1978). Early optimal foraging models predicted that foraging birds would never exhibit partial preferences for any encounter with food (Pyke et al., 1977). However, Great Tits have been shown to exhibit preferences that sometimes cause them to reject profitable food locations for less profitable ones (Getty & Krebs, 1985). The researchers believe that the birds showed a lag in responding to newly available food item. They also argued that the value of a given food item might change over time such that a non-optimal location should be sampled periodically as a "just in case" type of safety measure (Rechten et al., 1983). It is possible that the chickadees in our task were using this kind of preference, particularly in Experiment 1 where the cost of searching many locations was very low. In Experiment 3 it is likely that chickadees in the Win-stay condition used their preference for the "sure thing" (Barkan, 1990) by choosing the same locations across phases and trials in the task. Partridge (1976) discovered that Great Tits individually develop different preferred feeding methods where one individual prefers a particular pattern, and Sherry and Galef (1984) have also found evidence of these preferences in birds. This suggests that the search preferences in the current study are not surprising.

It is possible that this study did not accurately reflect win-shift and win-stay behavior specific to chickadees. Future studies will consider using different food rewards, such as mealworms, to increase incentive. Although chickadees forage on animal and vegetative matter, they tend to forage on animal matter, like insects, 70% of the time in the wild and on vegetation, like seeds and fruit, 30% of the time (Bent, 1946). Therefore, using animal matter as a food reward might provide greater incentive for subjects. Future studies should also consider using two-phase procedures in chickadees and the context in which chickadees are placed in-between phases. For instance, birds may associate their home cage with a replenishing process in the apparatus (every time they leave their home cage the rewards re-set). Therefore, future studies will use a cage of different context for the short period in between phases. Context could also be manipulated to distinguish between Phase A and B. Manipulations of context with rats have been successful in serving as a cue to subjects (Roberts et al., 2016). Lastly, another future experiment should involve separating win-shift and win-stay learning from spatial memory. I will use two trees, one that always replenishes (to promote a win-stay strategy) and one that always depletes (to promote a win-shift strategy) to see if chickadees are able to engage in this kind of task.

Our final possible explanation for the present results is that the Win-shift/Win-stay contingency is not an accurate measure of spatial search across species. Birds forage differently from the common Win-shift/Win-stay rodent demonstrator in a radial arm maze. Birds forage on a horizontal and vertical plane that cannot be compared to rodents. Perhaps the contingency is not measuring what we think it is, since studies with other species of birds such as the Rainbow Lorikeet (Sulikowski & Burke, 2011) and Noisy Miners (Sulikowski & Burke, 2012) have found no win-stay or shift preferences. The Rainbow Lorikeet is a facultative nectarivore that shows no bias for either the win-stay or win-shift contingencies (Sulikowski & Burke, 2011). However, Regent Honeyeaters are nectar-feeding birds that do avoid previously rewarded locations at short intervals and only return to them after long ones (Burke & Fulham, 2003). It is likely that experience plays an important role in foraging for different species and that in any case, foraging in any wild bird is an extremely complex, dynamic process that may never fit well with sweeping general models (Lucas, 1987).

3.5 References

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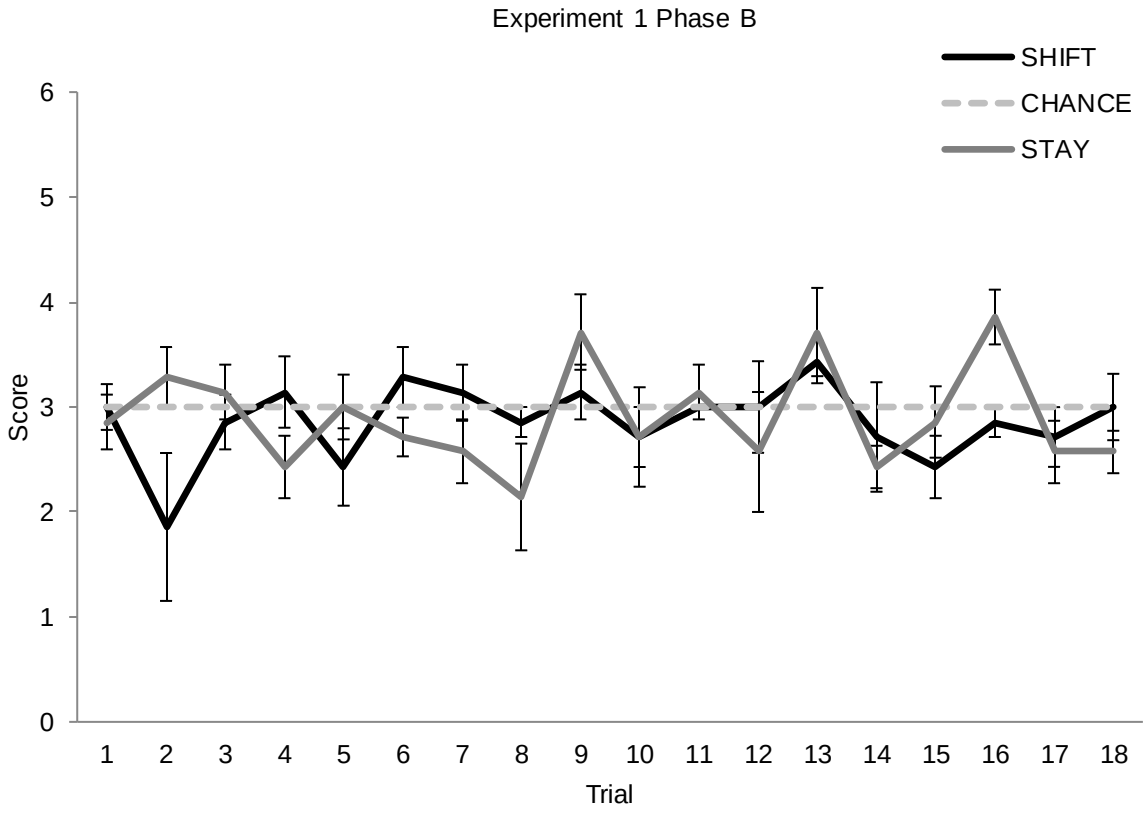

Figure 3.1. Performance of the Win-stay and Win-shift groups in Phase B of Experiment 1. Birds searched until all six baited locations were found or until 10 minutes had elapsed, whichever came first. Error bars represent standard errors of the mean.

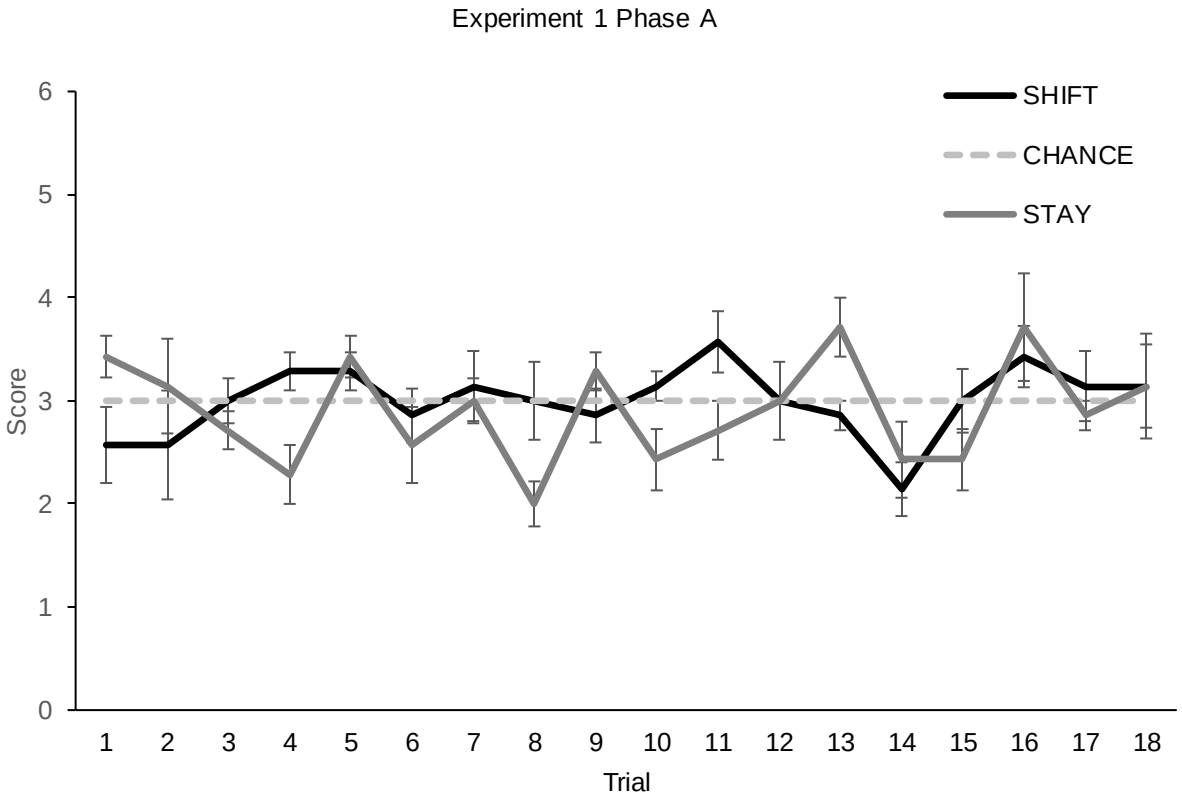

Figure 3.2. Performance of the Win-stay and Win-shift groups in Phase A of Experiment 1. Birds searched until all six baited locations were found or until 10 minutes had elapsed, whichever came first. Error bars represent standard errors of the mean.

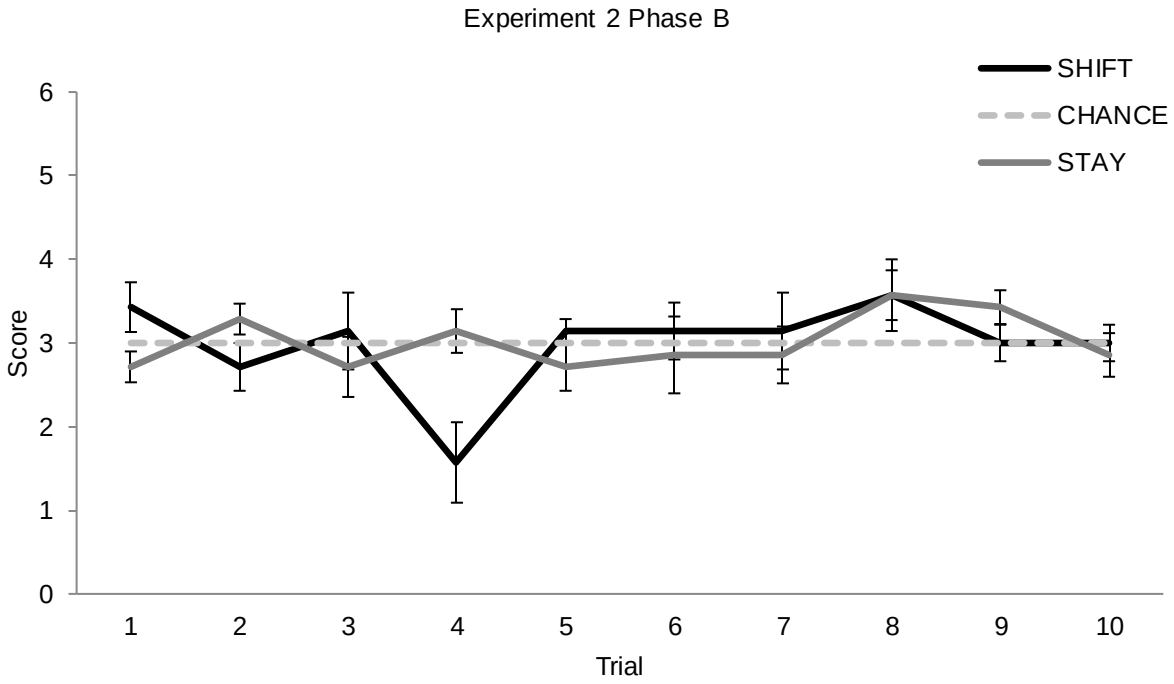

Figure 3.3. Performance of the Win-stay and Win-shift groups in Phase B of Experiment 2. Birds searched until six searches were made or until 10 minutes had elapsed, whichever came first. Error bars represent standard errors of the mean.

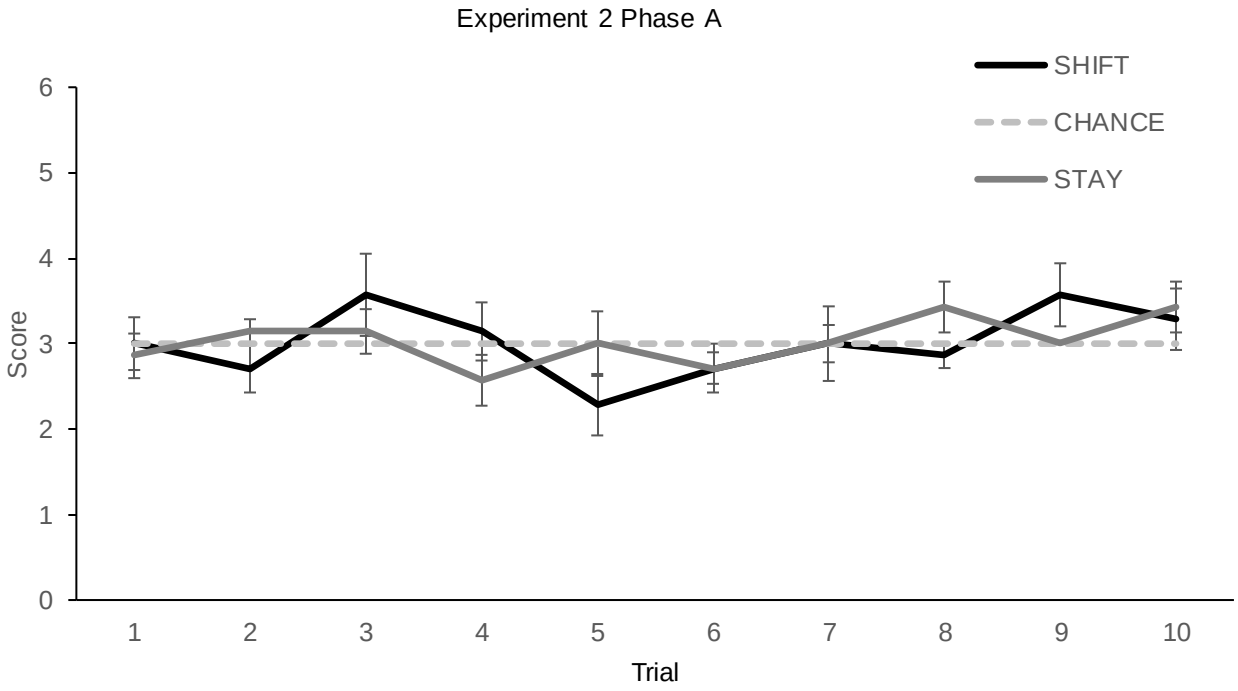

Figure 3.4. Performance of the Win-stay and Win-shift groups in Phase A of Experiment 2. Birds searched until six searches were made or until 10 minutes had elapsed, whichever came first. Error bars represent standard errors of the mean.

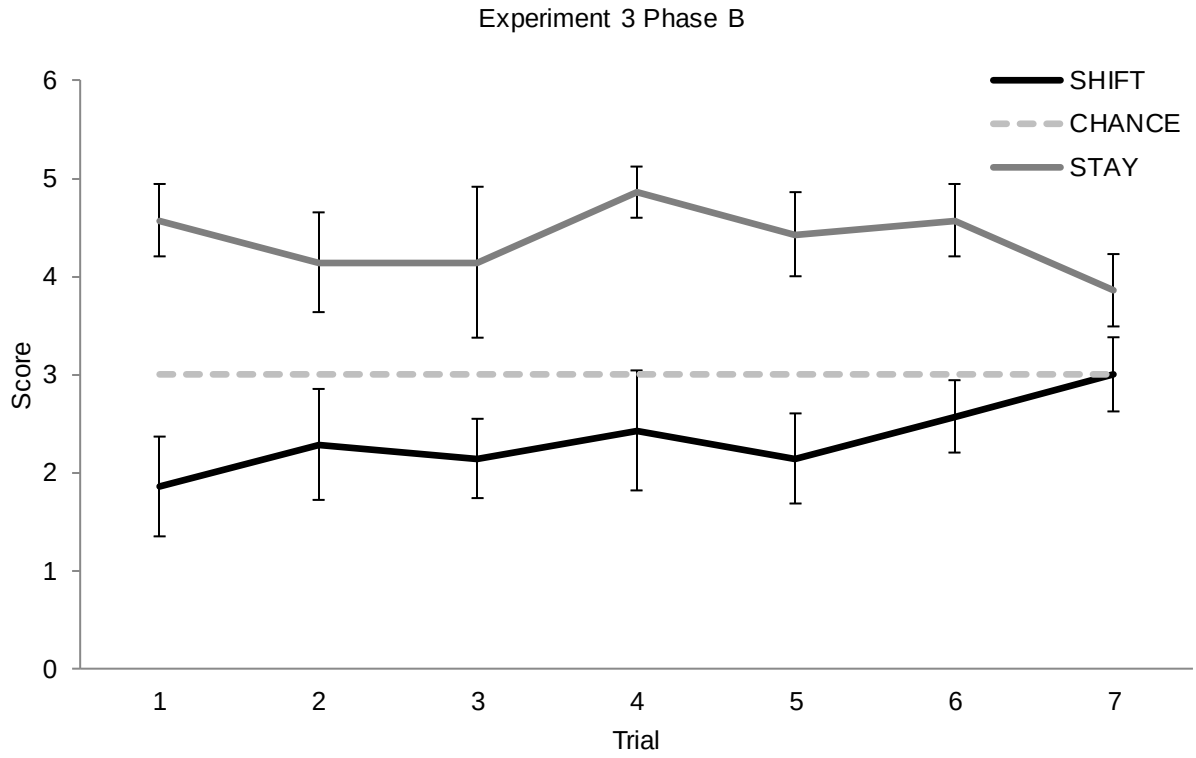

Figure 3.5. Performance of the Win-stay and Win-shift groups in Phase B of Experiment 3. In Phase A all 12 locations were baited. Birds searched in Phase A until they had taken food from any six locations. In Phase B, those same locations contained food for the Win-stay group. In the Win-shift group, only the 6 locations not visited in Phase A contained food. Birds were allowed only 6 searches in Phase B. Error bars represent standard errors of the mean.

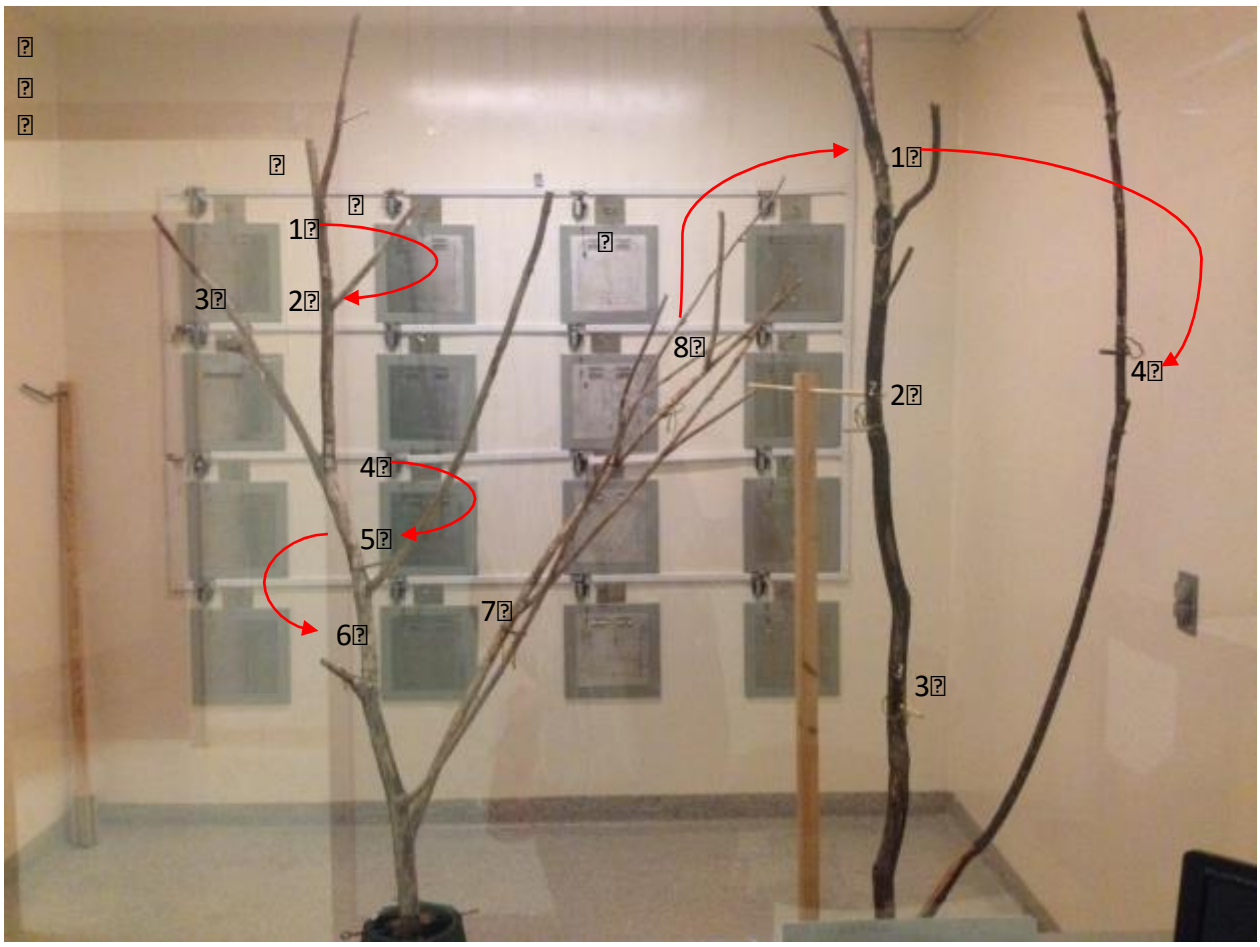

Figure 3.6. A diagram of significant transitions while searching. Birds frequently searched holes that were close in spatial arrangement. For example, after searching Tree 1 hole 1, birds would search Tree 1 hole 2, regardless of reinforcement, perhaps because the cost of doing so is low.

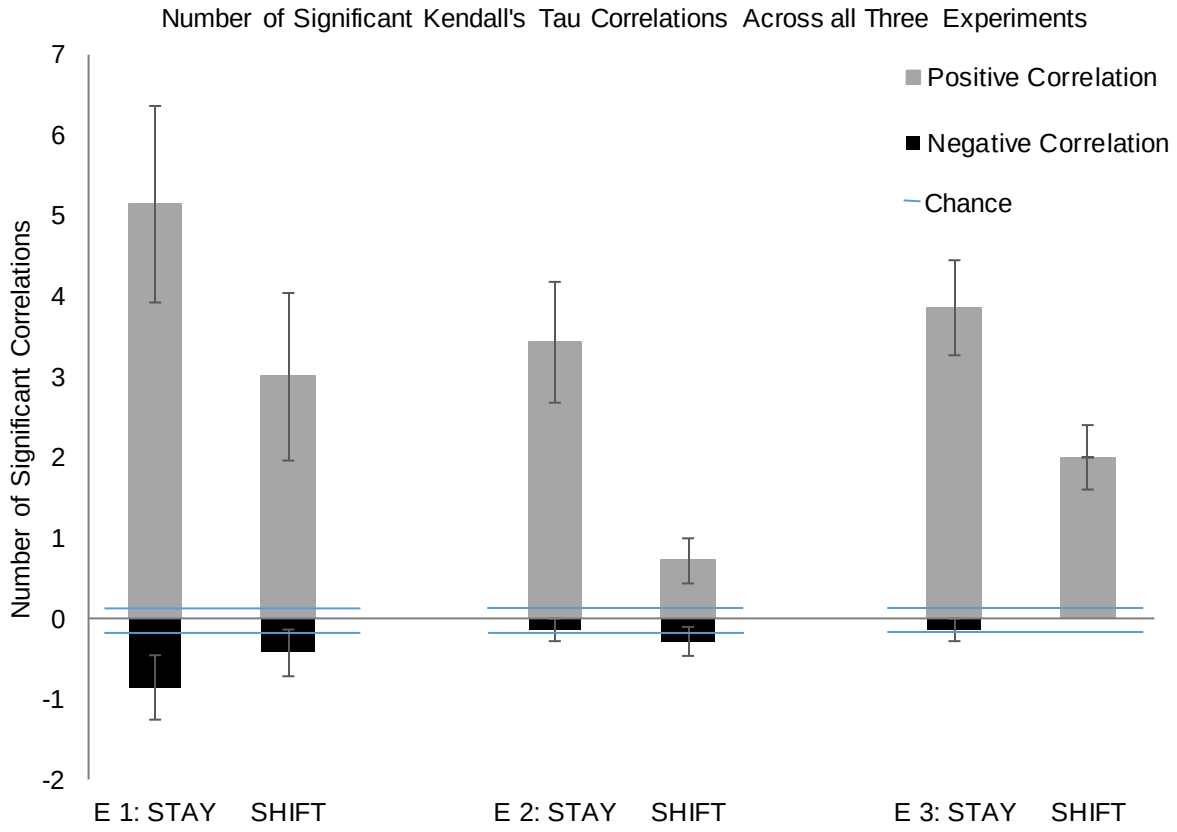

Figure 3.7. Significant Kendall's Tau correlations between locations searched in Phases A and B across all three experiments. Win-stay birds show more significant correlations between phases than Win-shift birds. Note that experiment 1 contained 18 trials, experiment 2 contained 10 trials, and experiment 3 contained 7 trials. Errors bars represent standard errors of the mean. Chance was calculated to be equal to +/- 0.1490 in experiment 1, +/- 0.0827 in experiment 2, and +/- 0.1177 in experiment 3.

Table 3.1 *Independent samples t-tests used to determine differences in positive and negative Kendall's Tau correlation means from chance across all three experiments for Win-Shift and Win-Stay birds.*

Correlation	Experiment	*df*	*t*	*p*
		Win-Shift		
Positive	1	6	2.72	0.03
	2	6	2.21	0.07
	3	6	4.45	0.004
Negative	1	6	-0.94	0.38
	2	6	-1.10	0.31
	3	N/A	N/A	N/A
		Win-Stay		
Positive	1	6	4.08	0.006
	2	6	4.45	0.004
	3	6	6.40	0.001
Negative	1	6	-1.80	0.13
	2	6	-0.42	0.69
	3	6	-0.59	0.58

Note. Significant differences were found for all postive correlations, excluding Win-Shift birds in Experiment 2. No significant differences from chance were found from negative correlations. Win-Shift birds in Experiment 2 made no significant negative correlations, and therefore, *t*-tests could not be calculated.

4.1 STUDIES OF ADULT HIPPOCAMPAL NEUROGENESIS AND WIN-SHIFT/WIN-STAY SPATIAL SEARCH: CONCLUSIONS

Introduction

The function of adult hippocampal neurogenesis, spatial memory and win-shift/win-stay foraging strategies were studied in Black-capped chickadees. Chapter 2 details the experimental manipulation of neurogenesis via methylazoxymethanol acetate (MAM), and the resulting consequences for spatial working and reference memory. Chapter 3 details a more fundamental investigation of chickadee foraging strategies using a win-shift/win-stay design. The aim of the current project was to understand the influence of adult hippocampal neurogenesis on spatial memory for the locations of food and to understand foraging strategies in chickadees. Adult hippocampal neurogenesis and win-shift/win-stay behavior are cognitive adaptations to the high demands of food storage on spatial memory, and the depleting and replenishing nature of a food source, respectively.

4.2 Adult Hippocampal Neurogenesis and Spatial Working and Reference Memory

In Chapter 2, two groups of chickadees were tested in spatial working and reference memory tasks. The treatment group received Methylazoxymethanol Acetate, a neurotoxin that decreases hippocampal neurogenesis in chickadees. The reference memory task required subjects to remember six out of twelve locations in trees that consistently contained a food reward, and was followed by a reversal. The working memory task required subjects to retrieve a food reward from twelve different locations. Pattern completion, the process that differentiates similar memories during encoding, may have been reduced by decreasing neurogenesis because fewer new neurons could be recruited into memory circuits. The results indicate that a reduction in adult hippocampal neurogenesis had no effect on the performance of either a spatial working memory task or a well-learned spatial reference memory task. There was a possible indication during reversal that hippocampal neurogenesis may contribute to successfully differentiating similar spatial memories at the time of encoding, and that this may rely on new neurons involved in pattern separation. This effect was non-significant, but may repay further investigation.

I hypothesized that if adult hippocampal neurogenesis aids memory then MAM treated subjects would perform less well than controls, and if adult hippocampal neurogenesis disrupts memory than MAM treated subjects would perform better than controls. The results indicate that

MAM subjects reverse slower than controls which may demonstrate that neurogenesis aids memory, however this effect was nonsignificant. The Attribute Model of Memory proposed by Hunsaker and Kesner (2013) involves using an event based system for episodic short-term retrospective memories like working memory. The event based system is involved in encoding memories and pattern separation. In contrast, the knowledge based system for long term reference memories is involved in retrieval and pattern completion. Therefore, retrieval relies on reactivation of the patterns of cellular activation that occurred during encoding (Frankland, Kohler, & Josselyn, 2014). Encoding is dependent on pattern separation, distinguishing two memories by reducing the overlap of brain activation between them (Treves & Rolls, 1994). It is possible that a decrease in neurogenesis inhibits the acquisition of conflicting information about where foraged food can be found by disrupting pattern separation processes. Evidence from Chapter 2 would support the idea that new neurons are necessary to differentially encode small or weak changes derived from increasingly similar or interfering outputs (Deng et al., 2010). Similarly, it may be the case that young hippocampal cells mediate pattern separation and old hippocampal cells mediate pattern completion (Nakashiba et al., 2012). Therefore, new neurons could be responsible for pattern separation, and MAM could have prevented pattern separation processes from occurring as seen by the trend during the reversal for MAM treated subjects to reverse more slowly than control subjects. Decreased neurogenesis increases the proactive interference of new anterograde memories (Deng, 2009). Barnea and Pravosudov (2011) hypothesize that blocking neurogenesis results in impaired spatial memory when cues to be remembered have little spatial separation, but not when cues have large spatial separation. The reference memory reversal in Chapter 2 occurred directly after the reference memory trials. Therefore, it is also possible that MAM treated subjects came to perform as accurately as control subjects only as trials continued.

It is also possible that significant working or reference memory deficits were not observed in Chapter 2 because subjects had a neurogenic reserve of new neurons ready to be integrated when there was a need for new learning (Kempermann, 2008). The neurogenic reserve hypothesis states that a reserve of new neurons allows the brain to be flexible when new information is being learned. Learning deficits occur only when the reserve of ready neurons depletes. Both explanations, that new neurons are necessary for pattern separation, and that there exists a Neurogenic Reserve, would adequately explain the current study's findings.

It is also possible that decreased neurogenesis does not influence a learned retrograde spatial working or reference memory (Frankland et al., 2014). As supported by our reference memory test findings, a decrease in neurogenesis caused less forgetting and pattern completion of retrograde memories comparable with controls. Decreased neurogenesis protects existing memories and acquisition of new information that conflicts with previously stored information may be impeded if neurogenesis is reduced after original learning. MAM subjects reverse slower than controls, so a decline in neurogenesis increases proactive interference of new anterograde memories. Similar evidence has been found in rodent studies where decreased neurogenesis impairs anterograde memory formation (Deng, 2009). New neurons are required to avoid interference when new information is being learned (Wiskott et al., 2006; Frankland et al., 2014). The present study's findings also suggest that if neuronal changes are occurring near established synaptic circuits, they coexist with, rather than alter those synaptic circuits (Frankland et al., 2014). It is also important to consider that the effect of reduced neurogenesis may be dependent on a number of factors such as; age at the time of neurogenesis reduction (Martinez-Canabal, 2012), the number of neurons targeted (Ko et al., 2009), the maturational stage of the targeted neurons at the time of learning (Gu et al., 2012), and the type of behavioral task used to assess learning and memory (Shors et al., 2002).

In Chapter 2, it was hypothesized that if neurogenesis aids memory, MAM treated subjects would perform less well than controls. If neurogenesis disrupts memory, MAM treated subjects would perform better than controls, due to decreased interference by new neurons. Therefore, the present work concludes that neurogenesis no plays no role in the working and reference memory tasks examined. The mechanism by which neurogenesis may aid memory is unclear. Whether new neurons are responsible for, or merely contribute to pattern separation, or whether there exists a neurogenic reserve are potential possibilities.

4.3 Win-shift and Win-stay Search Strategies in a Spatial Working Memory Task

In Chapter 3, two groups of Black-capped chickadees were tested in a spatial memory task to determine their spontaneous foraging strategy and whether they could flexibly employ win-shift or win-stay strategies depending on reward contingencies. Chickadees searched for food rewards in Phase A, and after a short interval, searched again in Phase B, with reward contingencies in Phase B reinforcing either a win-shift or win-stay strategy. The number of

searches they were allowed to make in Phase B varied across three experiments. Experiment 1 consisted of eighteen trials. In Phase A birds searched until they found the six pseudo-randomly determined baited locations, or until 10 min elapsed. In Phase B birds searched until they found six baited locations, which were either the same locations as in Phase A (for the Win-stay group), or the six locations not baited in Phase A (for the Win-shift group). Experiment 2 consisted of ten trials. In Phase A birds searched until they found the six pseudo-randomly determined baited locations, or until 10 min elapsed. In Phase B birds were allowed to search only six locations, regardless of whether or not they contained a reward. The purpose of restricting the number of locations birds were allowed to search was to increase the bird's motivation to choose correctly by imposing a limited number of searches as a cost. Again, the locations containing a food reward in Phase B were either the same locations as in Phase A (for the Win-stay group), or the six locations not baited in Phase A (for the Win-shift group). Lastly, Experiment 3 consisted of seven trials. In Phase A all twelve locations were baited. Birds were then allowed to search six locations and consume the seed fragments before the lights were turned off and they were sent back to their home cage. In Phase B birds were permitted to make only six choices as in Experiment 2. The correct locations to find a food reward in Phase B were the same locations as those they chose in Phase A (for the Win-stay group) the six locations not baited in Phase A (for the Win-shift group). Chickadees showed a win-shift strategy within each phase, but did not employ either a win-shift or win-stay strategy between phases, regardless of reinforcement. While chickadees successfully employ a win-shift strategy within a foraging bout, they appear indifferent to the renewal and depletion properties of food sources over a longer time scale.

Chickadee search strategies may be insensitive to changes in reward contingency as manipulated by these experiments, even though they forage on foods likely to promote both strategies. The win-shift/win-stay paradigm relies on hippocampal functions such as spatial working and reference memory, however, the limited cost of using a random search strategy may have provided insufficient incentive to employ a more effortful foraging strategy involving spatial memory. Location preferences were stronger than reward contingencies, suggesting that the birds relied on a motor pattern when searching for food rewards. Despite, early optimal foraging models predicting that foraging birds would never show partial preferences for any encounter with food (Pyke et al., 1977), Great Tits have been found to exhibit individual feeding preferences in foraging that sometimes cause them to reject profitable food locations for less

profitable ones (Getty & Krebs, 1985; Partridge,1976), and Sherry & Galef (1984) have also demonstrated individual preferences in birds. Rechten et al. (1983) hypothesized that the value of a given food item might change over time, such that a non-optimal location should be searched periodically. This theory explains the behavior observed in Experiment 1 where birds were permitted to search in Phase B until they uncovered all of the food rewards. Chickadees also display a preference for "sure-things" that explains the results of Experiment 3 for birds in the Win-stay condition. However, it may be the case that the foraging of Black-capped chickadees is a complex and dynamic process that does not fit well with sweeping general models like the Win-shift and Win-stay paradigm.

Win-shift/win-stay behavior has been described as a cognitive adaptation to the depleting or replenishing nature of a food source (Sulikowski & Burke, 2011). Food storing species, like chickadees, use spatial memory to recover their caches and forage (Tomback, 1980; Smulders & DeVoogd, 2000). To establish strong spatial memories in chickadees, the perceptual and motor experience of finding food, carrying it to a location, and storing it there, may be necessary (Baker et al., 1998), because chickadees are significantly better at finding their own food caches, and even when they watch another conspecific cache food they rarely relocate it accurately. Although chickadee memory for cached food is much greater than memory for found food (Baker et al., 1988) chickadees rely on found food 80-85% of the time (Pravosudov, 1985) and therefore we must understand the mechanism of spatial memory for found food in order to understand the memory mechanism responsible for chickadee foraging. Understanding of the cognitive adaptations that allow high demands on spatial memory, and for the search and recovery of depleting and replenishing food, are closely linked to understanding the evolution of chickadee spatial memory.

In Chapter 3, it was hypothesized that because chickadees forage on foods that deplete quickly and foods that deplete slowly, they would respond to reward contingencies in a Win-shift/Win-stay spatial foraging task. It is unlikely that chickadees do not have the memory capabilities required for this task, since they can keep track of thousands of stored food locations, and therefore I conclude that the Win-shift/Win-stay paradigm does not accurately reflect the foraging of birds. Our subjects lacked incentive to overcome their preferential search patterns and respond to reward contingencies.

4.4 General Conclusions

Memory is studied extensively in birds and chickadees because of their spatial memory required for remembering the locations of cached food. However, memory is also important for chickadee foraging in general. In chickadees, reference memory for the stable features of an experience would include information about the distribution and abundance of food, while working memory for the changing features of an experience, would monitor the ongoing performance of foraging. The purpose of this thesis was to examine two aspects of memory in chickadees: 1) the possible involvement of adult hippocampal neurogenesis in working and reference memory in chickadee foraging, given that chickadees are known to have higher levels of adult hippocampal neurogenesis than some other birds (Hoshooley & Sherry, 2007); and 2) how working and reference memory are involved in foraging in two contexts, one in which food is consistently located in the same place (and should promote a Win-stay strategy), and one in which food once found at a location does not re-occur there (and should promote a Win-shift strategy).

I found that adult hippocampal neurogenesis in Black-capped chickadees may be an important contributor to pattern separation and the differentiation of similar memories at the time of encoding, but in general, decreased neurogenesis has no influence on the working or reference memory tasks used in this experiment. Black-capped chickadees also appear to win-shift within a single foraging bout, but fail to take previous experience into account between foraging bouts. It is likely that our design did not capture this kind of foraging behavior in birds, but it is also apparent that chickadees use preferential search patterns when foraging. Combined, this work leads to the conclusion that the cognitive adaptations in chickadees that allow for large spatial memory loads and knowledge of depleting and replenishing food sources is influenced by hippocampal processes, and that complex and dynamic behaviors should never be grouped in with sweeping general models.

4.5 References

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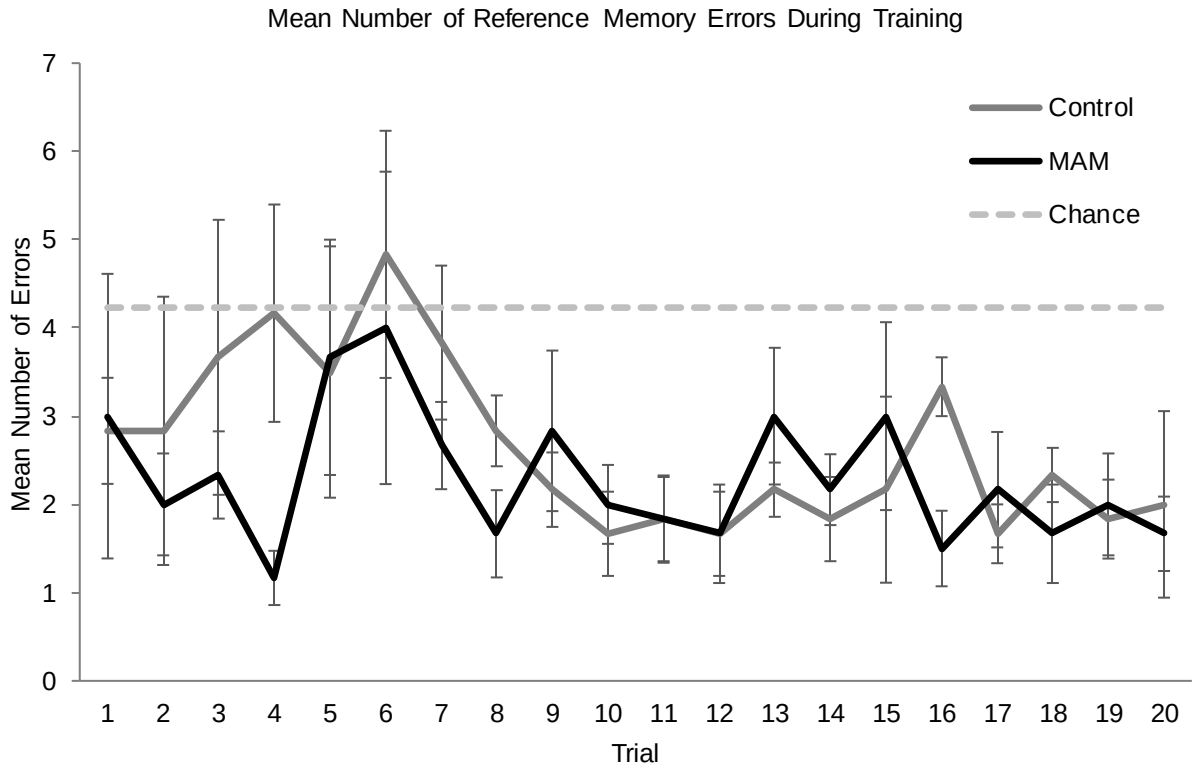
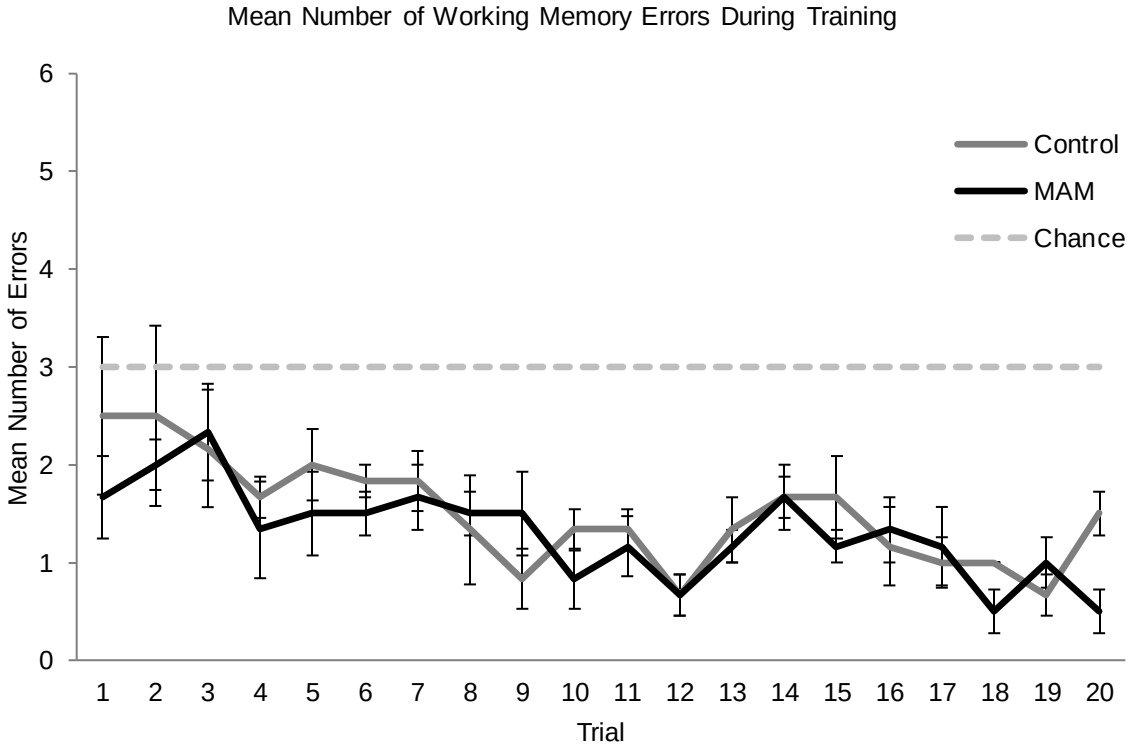
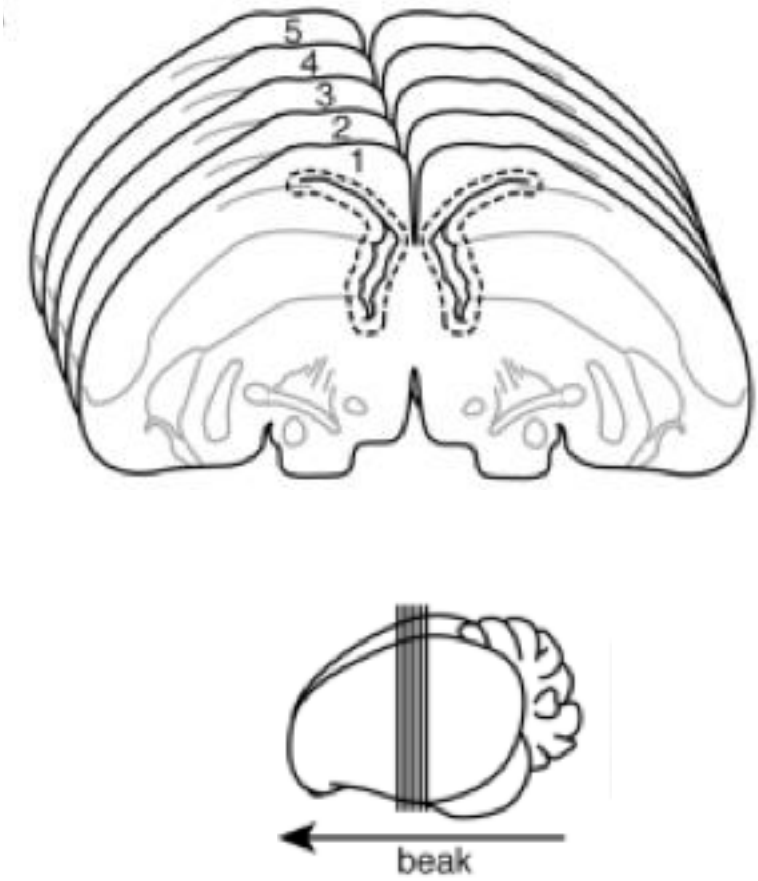
Appendices



Appendix A.2.1. Both the MAM, $t(19) = 16.13$, $p < 0.001$, and control, $t(19) = 12.41$, $p < 0.001$, groups performed significantly better than chance during training. There was no significant difference in accuracy between MAM or control subjects during training, $F(1, 10) = 0.75$, $p > .05$, $\eta_p^2 = 0.70$, but there was a significant main effect of trial, $F(5.69, 56.93) = 3.59$, $p < .001$, $\eta_p^2 = 0.26$ such that less errors were made as the number of training trials increased. There was no significant interaction between group and trial $F(5.69, 56.93) = 2.38$, $p > .05$, $\eta_p^2 = 0.06$. Error bars represent standard errors of the mean.



Appendix A.2.2. Both the MAM, $t(19) = 11.50$, $p < 0.001$, and control, $t(19) = 7.45$, $p < 0.001$, groups performed significantly better than chance. There was no significant difference in accuracy between MAM or control subjects during training, $F(1, 10) = 0.25$, $p > .05$, $\eta_p^2 = 0.02$, but there was a significant main effect of trial, $F(3.93, 39.31) = 4.03$, $p < .01$, $\eta_p^2 = 15.82$ such that less errors were made as the number of training trials increased. There was no significant interaction between group and trial $F(3.93, 39.31) = 0.912$, $p > .05$, $\eta_p^2 = 0.08$. Error bars represent standard errors of the mean.



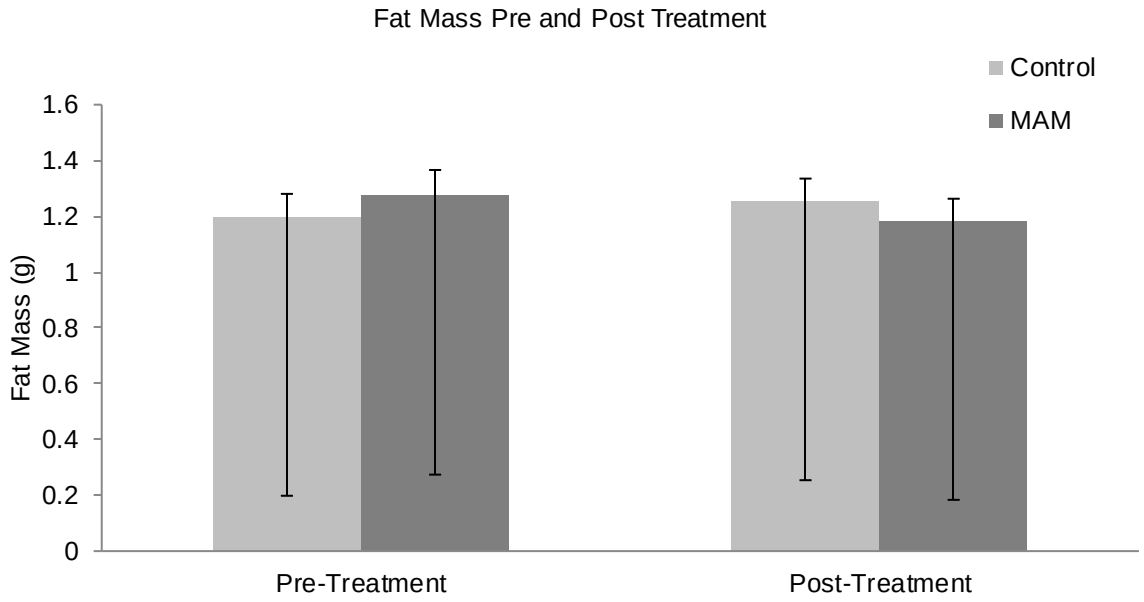
Appendix B.2. Brains were sectioned coronally (thickness = 40 µm). Once the subventricular zone (SVZ) was reached, as identified by whole-brain morphology, every tenth section in three alternating series was collected, until no sections containing hippocampus (HP) were remaining in each brain. Five of these slices were used for stereological cell counts. Image adapted from Hall, Delaney and Sherry (2014).

Appendix C.2.1

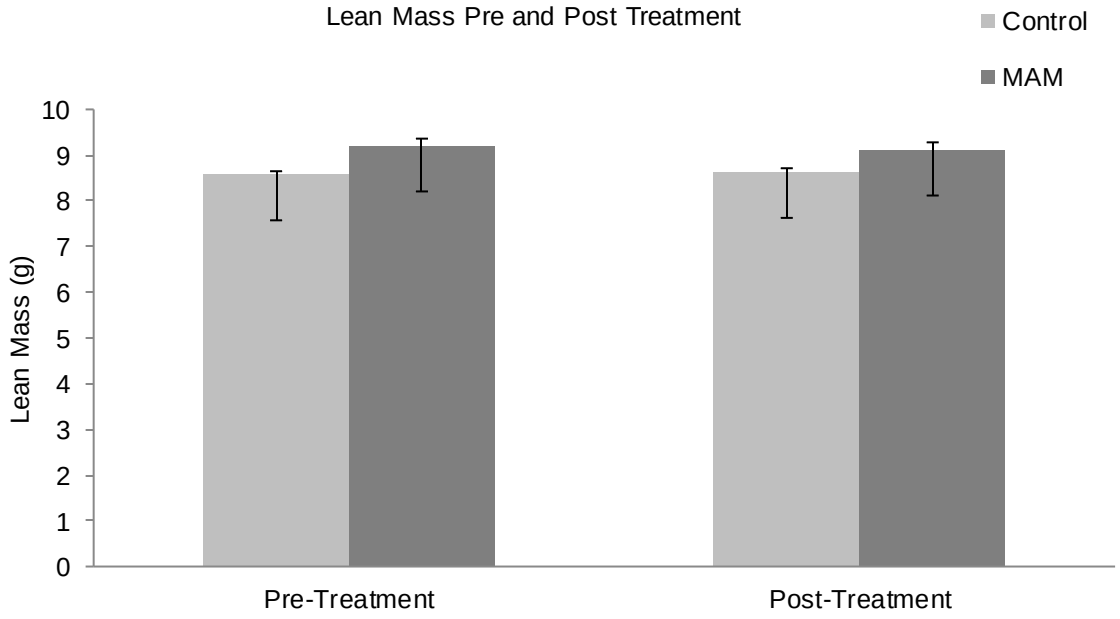
Paired sample t-tests of body composition before and after treatment of MAM or saline measured using Quantative Magnetic Resonance scanning

MAM Subjects	*df*	*t*	*p*
Fat Mass (g)	11	1.771	0.104
Lean Mass (g)	11	1.269	0.231
Free Body Water (g)	11	1.076	0.305
Total Body Water (g)	11	-4.424	0.680
Control Subjects	*df*	*t*	*p*
Fat Mass (g)	11	-0.986	0.345
Lean Mass (g)	11	-0.777	0.454
Free Body Water (g)	11	-1.326	0.212
Total Body Water (g)	11	-0.716	0.489

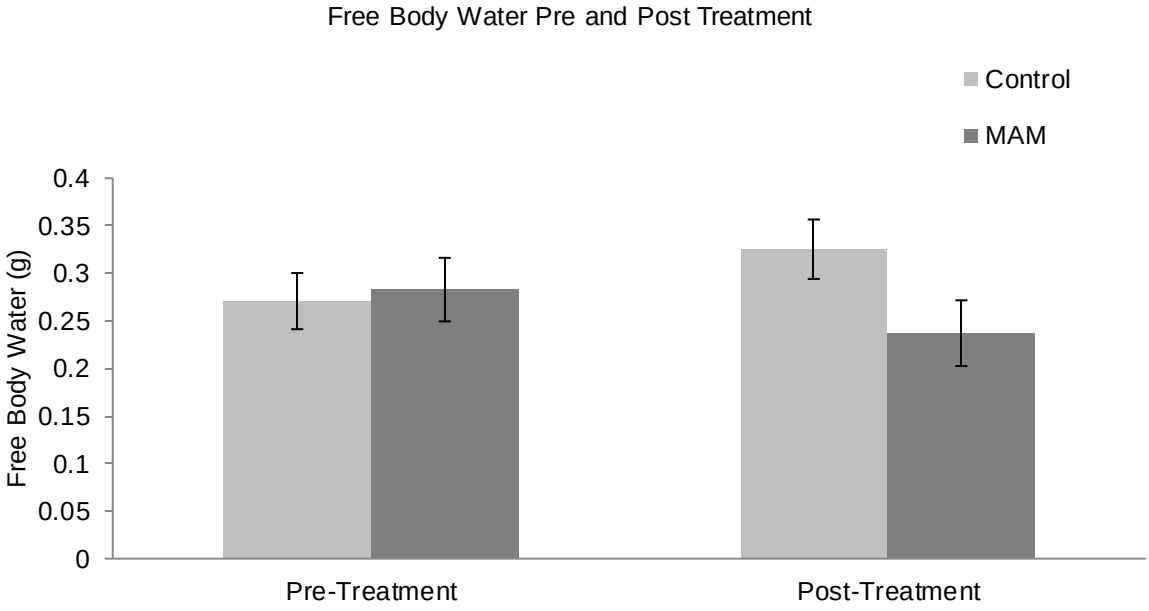
Note. Significant differences in neither control nor MAM subjects were found before or after MAM or saline administration.



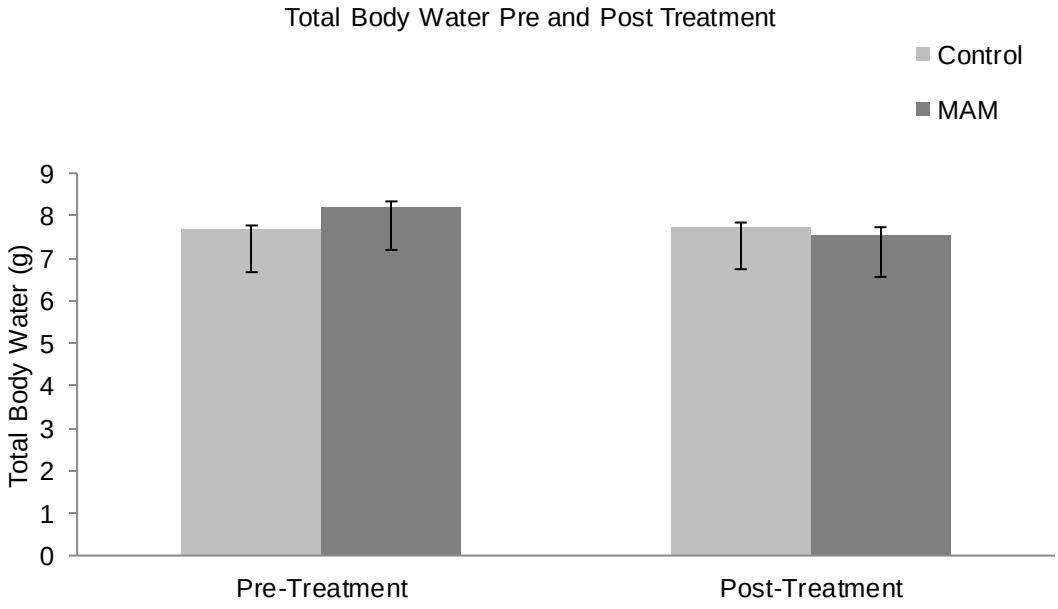
Appendix C.2.2. QMR results of fat mass (g) for control and MAM treated subjects before and after drug (MAM/ Saline) administration. Error bars represent standard errors of the mean.



Appendix C.2.3. QMR results of lean mass (g) for control and MAM treated subjects before and after drug (MAM/ Saline) administration. Error bars represent standard errors of the mean.



Appendix C.2.4. QMR results of free body water (g) for control and MAM treated subjects before and after drug (MAM/ Saline) administration. Error bars represent standard errors of the mean.



Appendix C.2.5. QMR results of total body water (g) for control and MAM treated subjects before and after drug (MAM/ Saline) administration. Error bars represent standard errors of the mean.

CURRICULUM VITAE

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AREAS OF RESEARCH

Working and reference memory, spatial memory, memory impairments, Alzheimer Disease, dementia, neurogenesis, hippocampus, and community mental health

PUBLICATIONS

Guitar, N. A., & Roberts, W. A. (2014). The interaction between working and reference spatial memories in rats on a radial maze. *Behavioral Processes, 112*, 100-107.

Roberts, W. A., **Guitar, N. A.**, Marsh, H., & MacDonald, H (2016). Memory systems in the rat: effects of reward probability, context, and congruency between working and reference memory. *Animal Cognition, 19*(3), 593-604.

CONFERENCE PARTICIPATION

Guitar, N. A. & Sherry, D. F. Adult Hippocampal Neurogenesis Aids Pattern Separation in Black-capped Chickadees. *23rd International Conference on Comparative Cognition.*
Melbourne, Florida, April 16-19th, 2016. Ron Weisman Outstanding Student Presentation Award.

***Guitar, N. A.**, Strang, C. G., Course, C. J., & Sherry, D. F.* Black-capped chickadees do not flexibly employ win-shift or win-stay strategies in a spatial working memory task. *23rd International Conference on Comparative Cognition.*
Melbourne, Florida, April 16-19th, 2016.

***Guitar, N. A.**, & Roberts, W. A.* The Interaction Between Working and Reference Spatial Memories in a Maze with Rats. *Canadian Society for Brain, Behavior and Cognitive Science (CSBBCS).*
Ottawa, Canada, June 5-7th, 2015.

***Guitar, N. A.**, & Roberts, W. A.* The Interaction Between Working and Reference Spatial Memories in a Maze with Rats. *22nd International Conference on Comparative Cognition.*
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TEACHING ASSISTANTSHIPS

Animal Cognition, Psychology 2210, The University of Western Ontario.
January 2016 – April 2016.

Animal Behavior, Psychology 3221, The University of Western Ontario.
September 2014 – December 2015.

Introductory Psychology, Psychology 1000, The University of Western Ontario.
September 2014 – April 2015.

INVITED LECTURES

"Studying memory in Black-capped Chickadee's"
Guest Lecture, Animal Cognition Psychology 2210, February 3rd, 2016.

"Memory and Memory Interactions"
Guest Lecture, Introductory Psychology 1000, November 27th, 2014.

"Why can't clients last the wait? Predictors for substance use waiting list attrition"
Guest Lecture, Psychology 3315 Addictions Theory and Research, September 9th, 2014.

"Why can't clients last the wait? Predictors for substance use waiting list attrition,"
Guest Speaker, Professional Network Forum, October 16th, 2014.

MEDIA

"hireWesternU" Campaign Video, the face of Community Engagement. Filmed February 12th, 2015. Available online March 5th, 2015 http://hirewesternu.ca

"Community Service Learning" Campaign Video, personal vignettes on community engaged learning experiences. Filmed February 12th, 2015.

SUPERVISORY EXPERIENCE

Work Study Research Assistant Student Supervisor, January 2015 – January 2016, Advanced Facility for Avian Research, London, Ontario.

RESEARCH ASSISTANTSHIPS

2015 Physiotherapist Research Assistant, Supervisor: Elizabeth Fox, Pursuits Health Management, London, Ontario

- Assisting in daily management of the clinic, conducting interviews, and administering surveys
- Conducting literature reviews on current treatment options for various ailments

2013-2014 Animal Cognition Lab, Supervisor: Dr. William A. Roberts, Western, London, Ontario

- Independently designed and implemented research projects
- Created an animal model of the interaction between working and reference memory commonly seen in memory impairments such as Alzheimer disease and dementia to allow for research on what behavioural or contextual therapies could be used to reduce that confusion

2013-2014 Quintin Warner House Research Assistant, Supervisor: Dr. Hinson**,** Missions Services, London, ON

- Worked professionally at an all-male residential substance use treatment facility to provide answers and solutions for why clients cannot withstand the long waiting period for treatment
- Implemented new techniques and approaches for patients currently on waiting lists to improve success rates

2011-2014 Canine Cognition Lab Research Assistant, Supervisor: Dr. William A. Roberts & Krista MacPherson, PhD. Candidate, Western, London, Ontario

- Data collection and recording in research on memory and cognition in canines
- Coordinating visits to the lab by dog owners from the community

AWARDS & SCHOLARSHIPS

External awards:

2016 Ron Weisman Outstanding Student Presentation Award, 23rd International Conference on Comparative Cognition, $200

2014 Royal Bank of Canada Community Professor Award, $1,000

2014 Royal Bank of Canada Collaborative Community Project Award, $1,000

2010 Loran Award Honours Citation

Internal awards:

2012 Western University Faculty of Social Science Alumni Award, $1,000

2012 Innovation in Leadership Award

2010-2014 Western University Continuing Scholarship, $10,000

2010-2014 Queen Elizabeth II Aiming for the Top Scholarship, $13,000